University of Alberta

Different Rhythmic Leg Movements in Human Infants May Share a Common Rhythm Generator

By

Kristin Elizabeth Musselman ©

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Abstract

This thesis addresses two central concepts in the neural control of locomotion. 1) Extension duration varies with cycle duration, whereas flexion duration is relatively invariant. 2) Neural circuits controlling walking control other rhythmic movements. We studied these concepts by observing human infants (aged 3 to 10 months) kicking, stepping and air-stepping. Video, electrogoniometry, force and surface electromyography data were collected. In study 1, we found that both phase durations of locomotion (extension and flexion) could vary with cycle length. The phase experiencing greater load varied more strongly with cycle duration. In study 2, infants showed similar interlimb coordination (alternate or synchronous) when kicking and treadmill stepping. Following practice of one interlimb coordination in weight-bearing, 3 infants changed their preferred coordination when treadmill stepping and to some extent when kicking. While we cannot say definitively that the same circuitry controls infant kicking, stepping and air-stepping, our research provides supporting evidence.

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Chapter 1: Literature Review

1.1 Overview of Thesis

Walking – it appears to be a relatively easy motor behavior learned when we are toddlers, but in fact the underlying neural control of walking is complex and has been the subject of study for over a century. This complexity becomes apparent after an injury to the central nervous system, when the ability to walk is compromised and re-learning this skill, if possible, is a difficult and lengthy process. Learning more about the neural control of walking will lead to more effective treatments and therapies for people struggling to regain their mobility and independence. The research in this thesis builds upon our current understanding of the neural control of locomotion, and more specifically, provides further insight into the neural control of stepping in human infants.

In this chapter we review the related work that motivated the research presented in this thesis. In section 1.2 we begin with a brief description of human locomotion, followed in section 1.3 by an introduction to central pattern generators (CPG) – what they are and an overview of some of the work in lower vertebrates that has fostered our understanding of their role in locomotion. Section 1.4 reviews evidence in favor of a CPG for locomotion in humans. The study of rhythmic movements in human infants is discussed in section 1.5. Sections 1.6 and 1.7 review our current understanding of how afferent and descending input, respectively, contribute to the neural control of walking. The idea of a multifunctional network, which is a neuronal circuit that is involved with more than one motor behavior, is discussed in section 1.8. The research in this thesis comments on whether the circuitry controlling infant stepping may also control other rhythmic

movements of human infants. Section 1.9 introduces the idea of an asymmetry in the control of extension and flexion phases of locomotion. Duration of the walking cycle, which involves alternating phases of flexion and extension of the legs, is modulated through the duration of the extension phase. Section 1.10 reviews the literature concerning the spinal control of interlimb patterns and section 1.11 discusses use-dependent plasticity in the spinal cord, with emphasis on work by Viala and colleagues (1986) who examined plasticity in conjunction with interlimb patterns in spinal rabbits.

Chapters 2 and 3 of this thesis each include brief introductions that highlight the portions of the related work mentioned above that are especially relevant to that study. In chapter 2 we study six rhythmic behaviors performed by human infants. For each rhythmic movement, we show that the phase experiencing the greatest resistance to movement (flexion or extension) varies more strongly with cycle duration. We suggest that the locomotor CPG may not have an inherent asymmetry in the control of extension and flexion phases, as was previously thought, but rather is a flexible system whose output is modulated by movement-related sensory feedback. In chapter 3 we compare the interlimb coordination adopted by human infants during non-weight-bearing (i.e., kicking) and weight-bearing activities. We show that infants express the same interlimb coordination in the two conditions, suggesting that the neuronal circuitries responsible for infant kicking and stepping may have shared components. We show that the type of interlimb coordination (i.e., alternate or synchronous) expressed by an infant is influenced by experience, demonstrating use-dependent plasticity in human infants. In

addition, we show that training of one type of interlimb coordination in weight-bearing may influence the coordination expressed in non-weight-bearing activities.

1.2 Introduction to Human Locomotion

Walking is a rhythmic movement requiring precise coordination of many muscles and joints to enable forward progression of the body's centre of mass. During walking, each limb alternates between periods of support (stance phase) and no support (swing phase). The bipedal, plantigrade walking of humans is unique in comparison to that of quadrupedal mammals in several respects. First, bipedal walking necessitates greater demands for equilibrium than quadrupedal walking. Secondly, at the beginning of the stance phase we extend our leg to contact the ground with our heel (heel strike) (reviewed in Forssberg, 1995; Capaday, 2002; Nielsen, 2003). Thirdly, early in the stance phase the dorsiflexors of the ankle contract eccentrically to lower the toes to the ground (reviewed in Nielsen, 2003). Finally, forward propulsion at the end of the stance phase is achieved by activation of our calf muscles (gastrocnemius-soleus complex), whereas quadrupeds and primates also rely on the quadriceps (reviewed in Forssberg, 1995; Nielsen, 2003). The difference in recovery potential following lesions of the central nervous system of bipeds and quadrupeds is also noteworthy. For example, cats can regain some stepping ability after a complete spinal cord transection (Barbeau and Rossignol, 1987), but humans with complete spinal cord injuries show minimal stepping recovery in spite of intensive training.

Despite these differences, human locomotion shares some characteristics with locomotion of lower vertebrates and even invertebrates. For example, as gait speed increases the durations of stance and swing change in a characteristic manner. In humans the duration of the stance phase varies strongly with gait speed whereas swing duration varies little if any (Grillner et al., 1979; Nilsson and Thorstensson, 1987). The same relationship between locomotor speed and stance/swing duration has been described for many terrestrial animals despite the fact that the rate of walking is radically different between species (Arshavskii et al., 1965; Delcomyn, 1971; Goslow et al., 1973; Grillner et al., 1979; Williams, 1981; Jacobson and Hollyday, 1982; Vilensky, 1983; Reilly and Delancey, 1997; Leblond et al., 2003; reviewed in Grillner, 1981; Hildebrand, 1985; Rossignol, 1996). Considering the similarities in locomotion across species, it is not surprising that the study of lower vertebrates and invertebrates has taught us a lot about the neural control of locomotion. We now know that locomotion is produced by complex interactions between spinal, descending (i.e., supraspinal), and sensory inputs, all of which are described in this chapter (reviewed in Nielsen, 2003; Zehr and Duysens, 2004).

1.3 The Role of Central Pattern Generation in Rhythmic Movements

In 1911, Brown published his influential paper documenting alternating activity of ankle flexors and extensors in a deafferented and spinalized cat. The preparation he used showed that the rhythmic behavior observed must have been generated by the spinal cord without descending or afferent input (Brown, 1911). Since the time of Brown, much research has been produced supporting the involvement of the spinal cord in the control of vertebrate locomotion.

Centrally-located neuronal circuits that produce rhythmic motor behaviors in the absence of afferent or supraspinal input are called central pattern generators (CPG). The CPG controls the timing of motoneuron activation, thus producing the basic alternating pattern of antagonist muscles needed for rhythmic movement (reviewed in Marder and Calabrese, 1996; Dickinson et al., 2000). The role of sensory or descending input includes initiation/termination of the behavior and modulation of the motor output to meet environmental and task demands (reviewed in Armstrong, 1986; Marder and Calabrese, 1996; Grillner et al., 1997; Dickinson et al., 2000). There is direct evidence for the existence of a locomotor CPG in many insects and animals (reviewed in Pearson, 1993; Bässler and Büschges, 1998; Grillner et al., 1998; Dickinson et al., 2000; Delcomyn, 2004).

In vertebrates, the locomotor CPG is located in the spinal cord (reviewed in Grillner, 1981). Reduced preparations of the cat have greatly advanced our understanding of spinal-mediated locomotion in vertebrates. Adult cats with acute spinal transections demonstrated the ability to step with the hindlimbs on a treadmill (Grillner and Zangger, 1979; Barbeau and Rossignol, 1987). This stepping did not occur spontaneously, but was induced by administration of noradrenergic or serotonergic agonists (Grillner and Zangger, 1979; reviewed in Nielsen, 2003), or manual stimulation of the perineal region or tail (Barbeau and Rossignol, 1987). Cats with chronic spinal injuries have also demonstrated treadmill stepping following intensive training (Grillner and Zangger, 1979; Eidelberg et al., 1980). The recovery of weight-supported treadmill stepping in spinalized cats appears to be age dependent, as cats spinalized at two weeks of age

showed greater improvements in stepping than cats spinalized at 12 weeks old (Smith et al., 1982). These preparations have allowed the study of spinal-mediated locomotion in the absence of supraspinal influences.

The strongest evidence for a CPG for locomotion comes from fictive preparations, which involve decerebration or spinalization of an animal in combination with deafferentation or immobilization (achieved pharmacologically) (Grillner and Zangger, 1979). In these preparations the spinal cord is isolated, as all supraspinal and sensory input is eliminated. Rhythmic activation of hindlimb muscles that resembles locomotion has been recorded in fictive preparations of the cat (Grillner and Zangger, 1979) and rodents (Kudo and Yamada, 1987; reviewed in Kiehn and Butt, 2003) thus demonstrating with certainty the presence of a locomotor CPG in these animals.

1.4 Spinal Contributions to Locomotion in Humans

Do humans have a locomotor CPG? Studies involving primates, humans with spinal cord injuries and human infants (section 1.5) have provided some supporting evidence. Fedirchuk and colleagues (1998) investigated the effects of several drugs, all known to elicit rhythmic locomotor activity in other mammals, in marmoset monkeys who had been decerebrated, paralyzed and acutely spinalized. Through hindlimb nerve recordings they observed rhythmic alternating activity of ipsilateral flexors and extensors and/or rhythmic alternating activity of ipsilateral and contralateral flexors following administration of clonidine or excitatory amino acids (such as N-methyl-D-aspartate (NMDA)). Their results provided evidence of spinal-generated motor outputs that

resembled locomotion in primates, yet these outputs proved more difficult to elicit in marmoset monkeys than in other mammals (L-3,4-dihydroxyphenylalanine (L-dopa), noradrenaline, and serotonin all failed to elicit spinal activity in the marmoset monkey). Although marmosets are closely related to humans from an evolutionary perspective, there are undeniable differences in the two walking systems that limit the usefulness of primate models for the study of human locomotion (for example we walk bipedally, while primates walk quadrupedally). Mori et al. (1999a, 2001) trained the Japanese monkey (*M. fuscata)* to walk bipedally on a treadmill, thus allowing comparison of bipedal walking between lower primates and humans. They reported some similarities, such as comparable ranges of motion of lower limb joints and the presence of push-off at the end of stance (Mori et al., 1999a). They also demonstrated that monkeys trained to walk bipedally adopt similar strategies as humans for the clearance of obstacles when walking (Mori et al., 2001). However, bipedal walking in monkeys showed notable deviations from human walking, such as a lack of heel strike at the beginning of stance (Mori et al., 1999a). Thus, the usefulness of primates for the study of human locomotion is limited.

In humans it is impossible to study rhythmic walking-like movements that are free from both descending and sensory input. However, the spinal-injured population has enabled study of rhythmic leg movements with limited descending input. Early reports of spontaneous stepping-like movements in individuals with clinically complete spinal cord injuries were made by Holmes (1915) and Kuhn and Macht (1948). Many years later Calancie and colleagues (1994) provided detailed description of involuntary movements

that resembled stepping in an individual with a chronic incomplete spinal cord injury. The movements appeared after the subject started a series of strengthening and walking exercises. The movements were elicited when the subject was lying supine with his hips extended. The rates of movement and the muscle activity recordings from the lower extremities resembled stepping motor patterns, and the authors suggested a central pattern generator for stepping was being observed (Calancie et al., 1994). However, since the subject had sustained an incomplete injury it is possible that input from spared descending tracts contributed to the movement. Bussel and colleagues (1996) documented rhythmic muscle activity in individuals with clinically complete spinal cord injuries. In addition they described parallels between the spinal networks of spinal-injured individuals and that of the dihydroxyphenylalanine (DOPA) network in animals – a network known to be related to the locomotor CPG in lower vertebrates (Bussel et al., 1996). For example, following electrical stimulation of the flexor reflex afferents, humans with spinal injuries showed a long latency flexor reflex in the ipsilateral flexors, just like cats with acute spinal injuries who had received DOPA (Bussel et al., 1996). Stepping behavior was elicited in individuals with chronic complete spinal cord injuries through epidural stimulation of the posterior structures of the lumbar cord, demonstrating that walking-like movements were possible without influence from the brain (Dimitrijevic et al., 1998). Another indication of spinal-controlled stepping in humans comes from reports of alternating leg movements surrounding the period of brain death (Hanna and Frank, 1995).

1.5 Rhythmic Movements in Human Infants

Throughout the first year of life, human infants perform rhythmic stepping movements when they are moved over a stationary surface or held erect over a moving surface, such as a slowly moving treadmill belt (André-Thomas and Autgaerden, 1966; Forssberg, 1985). This stepping behavior develops early in humans, as it does in other animals (reviewed in Sillar, 1994; Nishimaru and Kudo, 2000). Human fetuses have been documented to make stepping-like movements in utero at 10-14 weeks gestation (De Vries et al., 1984). Spinal and/or brainstem circuitries are believed to be responsible for infant stepping since this type of behavior has been reported in anencephalic infants (Peiper, 1961). Also, descending input from motor centres in the brain is weak at this age. Autopsy (Yakovlev and Lecours, 1967; Brody et al., 1987) and electrophysiological (Eyre et al., 1991; Khater-Boidin and Duron, 1991; Szelenyi et al., 2003) findings suggest that myelination of the corticospinal tract is not close to completion until about two years of age. The corticospinal tract is an important descending pathway for locomotion in humans (see section 1.7).

Formation of the human spinal cord begins early in prenatal development – the neural plate appears by the third gestational week (reviewed in Altman and Bayer, 2001). The first trimester is characterized by considerable growth of cord gray matter while the second and third trimesters involve expansion of white matter, myelination of fibre tracts (corticospinal tract excluded) and an increase in glial cells in the spinal cord (reviewed in Altman and Bayer, 2001). Growth of the corticospinal tract occurs throughout the second and third trimesters in a rostrocaudal direction (Humphrey, 1960). Similarly, myelination

of the corticospinal tract occurs rostrocaudally, beginning at about four months post-birth in the cervical cord and reaching completion around two years of age (Yakovlev and Lecours, 1967; Brody et al., 1997).

Several kinematic differences have been reported between infant and adult stepping. Immature infant stepping is digitigrade with hyperflexed hips and knees, synchronous flexion and extension of the lower limb joints, and dragging of the foot on the ground during swing (Forssberg, 1985; reviewed in Yang et al., 2004). These unique characteristics of infant stepping may result from the lack of, or limited, descending inputs. Yang and colleagues have studied infant stepping for many years, and have provided sufficient evidence to suggest that infant stepping is a highly organized behavior and not solely due to segmental reflexes (reviewed in Yang et al., 2004). They have demonstrated that infants utilize the sensory input received during stepping in a selective and functional way (reviewed in Yang et al., 2004). Recently they studied infant stepping on a split-belt treadmill and showed that infant stepping can adapt to a twenty-fold difference in the speeds of the two belts, and that one limb can step forwards while the other limb steps backwards (Yang et al., 2005). This implies that the putative pattern generators for each leg in the infant are independent to some extent; a finding also reported in quadrupeds (Forssberg et al., 1980). Yet, even at high speed differentials, the swing phase rarely co-existed in the right and left limbs, again an observation previously made in spinal cats (Grillner and Rossignol, 1978). This supports the idea of reciprocal inhibition between the flexor half-centres or flexor burst generators of the CPGs (Yang et al., 2005). Hence, the human infant is a useful model for studying spinal/brainstem-

mediated control of walking, but there are limitations associated with its use, which will be discussed in section 4.3.

1.6 The Role of Afferent Input in Locomotion

The role of afferent information during locomotion is to adjust the motor output according to the environment and/or the task at hand (reviewed in Pearson, 1993; Marder and Calabrese, 1996; Rossignol, 1996; Dickinson et al., 2000). There are three major groups of sensory information that may be received by animals during locomotion (reviewed in Dickinson et al., 2000). Two groups, directional sensors and equilibrium organs, are not addressed in this thesis. Directional sensors include sense organs that influence speed and direction (e.g., eyes) (reviewed in Dickinson et al., 2000). The inner ear is an example of an equilibrium organ, whose input assists to maintain proper body orientation during locomotion (reviewed in Dickinson et al., 2000). The third group, described below, includes movement-related feedback from muscle, joint and skin receptors (reviewed in Dickinson et al., 2000). Integrating afferent feedback from numerous sources results in appropriate modulation of the CPG motor output to achieve successful locomotion.

Movement-related afferent feedback can modulate the CPG output by influencing the transitions between phases (reviewed in Duysens and Van de Crommert, 1998). Sensory input exerts its influence either through CPG-controlled reflex pathways that synapse onto motoneurons (reviewed in Duysens and Van de Crommert, 1998), or directly onto CPG neurons that affect the timing of the pattern generator (Conway et al., 1987; Hiebert

et al., 1996; reviewed in McCrea, 2001). Muscle receptors, in particular the group I afferents (i.e., muscle spindles and Golgi tendon organs), are important contributors to locomotion. Muscle spindles respond to stretch of a muscle (reviewed in Matthews, 1981), while tendon organs respond to the force exerted by a muscle (Houk et al., 1971). Intact, decerebrate, spinal and fictive preparations of animals have consistently shown that input from group I afferents of extensor muscles during the stance phase of locomotion prolongs the ongoing ipsilateral extension phase and prevents transition into the swing phase (Duysens and Pearson, 1980; Conway et al., 1987; Pearson et al., 1992; Guertin et al., 1995; Whelan et al., 1995; Pang and Yang, 2000; reviewed in Whelan, 1996; McCrea, 2001).

Group I afferents of flexors also influence the step cycle (reviewed in McCrea, 2001). Treadmill walking in decerebrate cats has demonstrated that stretch of hindlimb flexor muscles during stance promotes ipsilateral flexion and therefore, the transition from stance to swing (Hiebert et al., 1996). Flexor activity was also enhanced following stimulation of flexor afferents of the hindlimbs of decerebrate cats during the swing phase (Lam and Pearson, 2001). Blocking hip flexion during swing, which likely resulted in increased muscle spindle and Golgi tendon organ activation, resulted in a prolongation of flexor activity (Lam and Pearson, 2001). Similarly, in mesencephalic locomotor region (MLR)-activated fictive preparations, stimulation of hindlimb flexor nerves during the swing phase at levels that activate group I afferents prolonged ipsilateral flexion (Perreault et al., 1995; Quevedo et al., 2000). However, flexor affterent stimulation during the swing phase at levels known to activate group II afferents caused a switch

from flexor to extensor activity (Perreault et al., 1995). Thus, muscle afferents signaling stretch and load can alter the motor output of the locomotor CPG. In fact, unloading of the extensor muscles at the end of the stance phase is thought to be a trigger for the transition to the swing phase (Duysens and Pearson, 1980; reviewed in Capaday, 2002).

An extended hip position is also an important factor in the regulation of the stance to swing transition of locomotion in spinal cats (Grillner and Rossignol, 1978), decerebrate cats (Hiebert et al., 1996), fictive locomotion in decerebrate cats (Pearson and Rossignol, 1991) and human infants (Pang and Yang, 2000). Which afferents contribute to this phenomenon (i.e., flexor muscle afferents detecting stretch and load or joint afferents, or both) is not clear (Grillner and Rossignol, 1978; Pang and Yang, 2000), but recent work suggests activation of hip flexor muscle spindles and Golgi tendon organs are involved (Lam and Pearson, 2001). It appears that inputs signaling hip position and load of the limb combine together to permit or prevent the transition from stance to swing during locomotion in human infants (Pang and Yang, 2000).

Cutaneous inputs influence locomotion in a phase-dependent manner (Forssberg et al., 1975; Bouyer and Rossignol, 1998; reviewed in McCrea, 2001). Activation of cutaneous afferents of the dorsum of the paw/foot during the swing phase promotes flexor activity; a behavior known as the stumbling-corrective reflex, whereas the identical stimulus applied during stance promotes activity of the extensors (Forssberg et al, 1975; Forssberg, 1979; reviewed in McCrea, 2001; Edgerton et al., 2004). During fictive locomotion in cats, induced by stimulation of the mesencephalic locomotor region (MLR), the same phase-

dependent response was seen following stimulation of a cutaneous nerve that innervates the dorsum of the paw (Guertin et al., 1995). Lam and colleagues (2003) showed that a human infant's response to a touch to the foot during stepping depends on the site of stimulation, the phase within which the stimulation was applied and the direction of stepping (i.e., forwards, backwards, sideways). A response to the stimulation was seen only when it could be perceived as interfering with the progression of locomotion (i.e., a flexion response was seen following touch to the dorsum of the foot but not the side of the foot during the swing phase of forwards stepping) (Lam et al., 2003). This finding demonstrates that the spinal/brainstem circuitry responsible for locomotion is capable of selectively gating the sensory input it receives (Lam et al., 2003; reviewed in Yang et al., 2004).

1.7 The Role of Descending Input in Locomotion

Descending input from cortical structures influences walking in many ways, such as initiating the behavior. Self-initiation of locomotion is not seen in spinal cats; instead drugs that act like the noradrenergic and serotonergic transmitters normally released by descending pathways are frequently used (reviewed in Duysens and Van de Crommert, 1998; Grillner, 2003). Supraspinal areas believed to be involved in initiating locomotion in the cat are the mesencephalic locomotor region (MLR) and subthalamic locomotor region (SLR) in the brainstem, since electrical stimulation of these areas can induce locomotion (reviewed in Armstrong, 1986; Whelan, 1996). There is some evidence pointing to a similar area in humans (Hanna and Frank, 1995). The MLR projects to the medial medullary reticular formation and then to spinal interneurons via the ventrolateral

funiculus (reviewed in Whelan, 1996). The descending path of the SLR is not as well known, but it may project to other brainstem areas, such as the medial reticular formation and MLR (reviewed in Whelan, 1996).

Other roles of descending input likely include maintaining balance during walking and adapting the movement to deal with environmental factors, such as uneven terrain or obstacles (reviewed in Drew et al., 2004). The cerebellum is important for refinement of motor acts as it is an integrator of afferent input from many sources (reviewed in Arshavsky et al., 1983; Grillner, 1985; Armstrong, 1986). Spinocerebellar and spinoreticulocerebellar tracts relay a copy (called an efference copy) of the CPG output to the cerebellum, which then adjusts the output as required via descending pathways that directly and indirectly connect to motoneurons (Arshavsky et al., 1984; reviewed in Grillner, 1985; Armstrong, 1986). The cerebellum is also capable of evoking locomotion in decerebrate cats. Mori and colleagues (1999b) reported that stimulation of midline cerebellar white matter produced fore- and hindlimb stepping movements when the cats were supported over a moving treadmill belt.

There are numerous tracts relaying motor-related information from the brain to the spinal cord, namely the vestibulospinal, reticulospinal, rubrospinal and corticospinal tracts. Once again, study of lower vertebrates has fostered our understanding of the descending tracts and their role in locomotion. Lesion studies in cats have identified descending tracts relevant to locomotion. Lesions to the ventrolateral tracts (reticulospinal and vestibulospinal) do not abolish locomotor ability, but do result in postural deficits

(Brustein and Rossignol, 1998). Locomotion in cats is also possible after interruption of the dorsolateral funiculi, but with characteristic gait deficits, the most visible being dragging of the paw throughout the swing phase (Górska et al., 1993; Jiang and Drew, 1996). The dorsolateral pathways consist of the corticospinal (also known as pyramidal) and rubrospinal tracts, which are involved in the control of distal musculature (reviewed in Grillner et al., 1997).

Although these observations are insightful, the descending control of locomotion in humans has several important differences from cats. A crucial descending tract for walking in humans is the corticospinal tract, which provides extensive direct monosynaptic connections between the motor cortex and motoneurons in the spinal cord (Schoen, 1964; Kuypers, 1973; reviewed in Nielsen, 2003). This allows the motor cortex to contribute directly to muscle activation (reviewed in Nielson, 2003). Lesions of the motor cortex or corticospinal tract have more severe consequences in humans than in other animals, suggesting the motor cortex and corticospinal tract play a greater role in the motor control of humans (Porter and Lemon, 1993; Nathan, 1994). It has been suggested that if a CPG for human locomotion exists, it likely relies more heavily on descending input than that of lower vertebrates (reviewed in Nielsen, 2003).

1.8 Multifunctional Networks

Neuronal circuits (i.e., CPGs) underlying motor behaviors are not discrete and independent, but rather systems of neurons that contribute to the generation of more than one behavior (reviewed in Marder and Calabrese, 1996; Dickinson et al., 2000;

Kupfermann and Weiss, 2001). Examples include tactile reflexes and voluntary leg movements (e.g., walking, searching and rocking) in the stick insect (Kittmann et al., 1996); respiratory pumping and the gill withdrawal reflex in the Aplysia abdominal ganglion (Wu et al, 1994); pyloric and gastric-mill rhythms in the stomatogastric ganglion of the crab (Weimann & Marder, 1994); flying and running in the dorsal giant interneurons of the cockroach (Ritzmann et al., 1980); and fictive respiration, swallowing and coughing in the decerebrate cat (Oku et al., 1994). These multifunctional networks rely on modulatory input, such as afferent or supraspinal input, to determine which motor pattern is expressed (reviewed in Marder and Calabrese, 1996).

Electrophysiological evaluation of network components has been used to identify motor behaviors sharing circuitry (Ritzmann et al., 1980; Oku et al., 1994; Weimann and Marder, 1994; Wu et al., 1994; Kittmann et al., 1996). However, this method of evaluation is not always viable due to the size and complexity of the nervous systems of many vertebrates. Instead, indirect methods have been used to identify which motor behaviors likely stem from a common neural network (Earhart and Stein, 2000b; reviewed in Bekoff, 1992). Earhart and Stein (2000b) found similarities in the kinematics and motor patterns of six behaviors of the turtle, concluding that there is likely a basic sharing of circuitry for all behaviors, with greater sharing for those that demonstrated similar motor patterns. Similarities in motor output and response to deafferentation of the hindlimbs in chicks prompted Bekoff and colleagues (Bekoff et al., 1987; reviewed in Bekoff, 1992) to hypothesize that one set of neural circuitry is modulated to produce hatching and walking behaviors.

Blends of behaviors also signal multifunctional networks. Blends can be manifested as hybrids or switches. A hybrid is a behavior that resembles two distinct movements - it is a mixture of kinematic and motor characteristics from the two original behaviors (reviewed in Stein, 2005). For example, Earhart and Stein (2000a) reported the existence of a scratch-swim hybrid in spinal turtles. A switch involves the smooth transition from one behavior to another with no breaks or discontinuities (reviewed in Stein, 2005). The presence of hybrids and switches suggests that the two original behaviors are produced by shared neuronal circuitry (Earhart and Stein, 2000a; reviewed in Stein, 2005).

Lam and Yang (2000) proposed that the same locomotor CPG controls infant stepping in the forwards, backwards and sideways directions. They reached this conclusion after observing that stepping in all directions responded in a similar way to increases in speed, and that there were no abrupt changes in motor or kinematic patterns as the direction of stepping was gradually changed (Lam and Yang, 2000). Human infants perform other rhythmic movements that appear similar to stepping. Infants commonly kick with their legs while lying on their backs for the first 10 months of life (Thelen, 1985). When held vertically in the air, infants can also air-step (i.e., step-like movements without the feet contacting a surface), although this behavior is seen less frequently than stepping or kicking (Yang et al., 1998). Is the same neural circuitry underlying the infant stepping response also involved with other lower extremity motor behaviors of infants, such as kicking and air-stepping?

1.9 Asymmetry in the Control of Extension and Flexion Phases of Locomotion

As walking speed increases, the duration of the step cycle shortens. We mentioned in section 1.2 that a universal characteristic of locomotion is that the extension/stance phase varies with changes in step cycle duration (i.e., gait speed), whereas the swing phase is relatively invariant (Arshavskii et al., 1965; Delcomyn, 1971; Goslow et al., 1973; Grillner et al., 1979; Williams, 1981; Jacobson and Hollyday, 1982; Vilensky, 1983; Nilsson and Thorstensson, 1987; Reilly and Delancey, 1997; Leblond et al., 2003; reviewed in Grillner, 1981; Hildebrand, 1985; Rossignol, 1996). This observation translates to experiments on fictive preparations as well. In fictive preparations of the cat the duration of the extensor bursts, but not the flexor bursts, changed with changes in cycle duration (Grillner and Zangger, 1979; Dubuc et al., 1988; Pearson and Rossignol, 1991; Leblond and Gossard, 1997). The combination of these observations has led researchers to believe that the locomotor CPG is programmed to adapt to changing cycle periods by mainly adjusting the length of the extension phase (reviewed in Grillner and Dubuc, 1988).

In contrast, one of the several experiments with fictive preparations of the cat performed by Grillner and Zangger (1979) showed flexor activity, rather than extensor activity, to vary with cycle length. This phenomenon has been reported for fictive preparations at least two other times (Douglas et al., 1993; Yakovenko et al., 2005). In fact, Yakovenko and colleagues (2005), when studying midbrain locomotor region-induced fictive locomotion in adult decerebrate cats, reported that variations in cycle length were associated most strongly with changes in flexor duration in 70% of preparations.

Extensor and flexor phase durations have been found to vary to the same extent with changes in cycle duration for fictive locomotion of spinal cats (Baker et al., 1984) and also for walking in chicks that have undergone deafferentation of the limbs (Bekoff et al., 1987).

Although there appears to be some discrepancy in the results of experiments involving fictive preparations, there have never been reports of anything but an extension asymmetry (i.e., changes in cycle length vary largely with changes in extension phase duration) for terrestrial walking. This asymmetry has even been reported for kicking in human infants (Thelen et al., 1981). A deviation from the extension asymmetry is seen when stepping occurs without contact of the feet/paws with a surface (i.e., air-stepping). Vilensky and colleagues (1989) analyzed air-stepping in healthy infant Vervet monkeys. They documented hindlimb powerstroke (i.e., extension of the limb) to be independent of cycle duration, and hindlimb returnstroke (i.e., flexion of the limb) to correlate highly with cycle duration (Vilensky et al., 1989). Similarly, Bradley and Smith (1988) reported longer tibialis anterior bursts than lateral gastrocnemius bursts in spinal kittens during air-stepping.

The above-mentioned inconsistencies in the literature have caused us to question whether the CPG for locomotion has an inherent asymmetry in the way extension and flexion phases are controlled. Knowing the strong influence of afferent feedback on the CPG output (reviewed in section 1.6), it may be possible that the locomotor CPG has no

inherent asymmetry, but shows an extension or flexion asymmetry depending on the sensory input received. This idea is developed and studied in chapter 2 of this thesis.

1.10 Spinal Control of Interlimb Coordination

The coordination of homologous limbs can be described by the timing relationship of the same event in two limbs during rhythmic movements. Interlimb coordination is commonly described as either out-of-phase (i.e., the two limbs move in alternation) or in-phase (i.e., the two limbs move in synchrony). Spinal circuitry is believed to control interlimb coordination (Forssberg et al., 1980; Field and Stein, 1997; reviewed in Grillner, 1981). The half-centre hypothesis for CPG organization, which is one of several hypotheses, views the locomotor CPG as a system of two half-centres (i.e., a flexion and extension centre) for each limb governed by mutual inhibition (Brown 1911, 1914). According to this hypothesis, the CPG regulates an alternate relationship between the limbs by reciprocal inhibition of the flexor half-centres, thereby preventing concurrent swing phases in both limbs (Brown, 1911, 1914; reviewed in Lundberg, 1980). However, spinal circuitry seems to have the capability to express both alternate and synchronous coordination. Field and Stein (1997) reported out-of-phase and in-phase hindlimb movements for caudal scratching in spinal turtles. Spinal cats were observed to step with an out-of-phase coordination at slow treadmill speeds and an in-phase pattern at high speeds (Forssberg et al., 1980). Viala and colleagues (1986) also reported both forms of interlimb coordination in spinal rabbits. We have observed human infants to show both coupling patterns when on the treadmill. Some infants step alternately, some hop with the two legs moving in synchrony, while others show a mixture of stepping and hopping.

Thus, the circuitry responsible for regulating alternate and synchronous coordination of the limbs appears to exist in the spinal cord.

The neonatal rat has increased our knowledge of the control of interlimb coordination at the spinal level (Kremer and Lev-Tov, 1997; Butt and Kiehn, 2003; reviewed in Kiehn and Butt, 2003). Some of the responsible circuitry has been located in the ventromedial lumbar spinal cord of the rat and is believed to include the commissural interneurons (Kremer and Lev-Tov, 1997; reviewed in Kiehn and Butt, 2003). Pharmacological studies identified that out-of-phase hindlimb movements involve glycinergic inhibitory pathways, while in-phase movements are mediated by weaker glutaminergic excitatory pathways (reviewed in Kiehn and Butt, 2003).

Do human infants show a preference for one type of interlimb coordination over the other when on the treadmill due to their experience (e.g., play habits)? In addition, if an infant demonstrates a preference for one type of coordination on the treadmill, does he/she express the same coordination when kicking? If stepping and kicking share underlying neural circuitry, the infants should show the same interlimb coordination when kicking and when on the treadmill. These ideas are addressed in chapter 3.

1.11 Use-dependent Plasticity in the Spinal Cord

Throughout life we learn new motor skills, or re-learn motor tasks following a neurological injury. The nervous system supports such learning through chemical and structural changes, referred to as plasticity. Neural plasticity in the motor cortex has been

studied with some success. The sensorimotor cortex can reorganize its body limb maps in response to experience or injury (Jenkins and Merzenich, 1987; Weiller et al., 1993; Pascual-Leone et al., 1994). The spinal cord is also capable of learning. When walking on a treadmill, spinal cats avoided an obstacle in their path by hyperflexing their joints during the swing phase, and this response remained for several steps after the obstacle had been removed (Hodgson et al., 1994). This is an example of short-term learning at the level of the spinal cord. Longer-term learning is also possible (reviewed in Wolpaw and Tennissen, 2001) and has been demonstrated by spinal cats that regain the ability to walk following locomotor training (reviewed in Edgerton et al., 2004). Wolpaw and O'Keefe (1984) demonstrated that the spinal stretch reflex in monkeys could be up- or down-trained (i.e., an increase or decrease in amplitude, respectively) by providing a food reward when the reflex amplitude was above or below, respectively, a criterion value. This asymmetry in the reflex remained after acute spinal transaction, indicating long-term changes in the spinal cord.

One of the many interesting characteristics of spinal plasticity is its use-dependent nature (Hodgson et al., 1994). Edgerton and colleagues (1997) demonstrated that spinal cats trained to step could successfully step on a treadmill but were unable to stand, whereas cats trained to stand did not regain the ability to step. When a cross-training paradigm was introduced (i.e., previously step-trained cats received only standing training, and previously stand-trained cats practiced stepping only) the animals acquired the ability to perform the newly trained task but lost the ability to perform the initially trained task (Edgerton et al., 1997). These results indicate that the spinal cord learns in a task-specific

manner. The underlying processes that enable spinal plasticity are not known, but in recent years researchers have discovered that activity causes an upregulation of neurotrophins, such as brain-derived neurotrophic factor and neurotrophin-3, in the spinal cord (Ying et al., 2003; Ying et al., 2005). Viala and colleagues (1986) also showed use-dependent plasticity in spinal rabbits following passive training rather than active training. Spinal rabbits were trained for twenty days in a motor-driven bicycle-like apparatus that permitted one of two hindlimb coordination patterns: 1) in-phase or synchronous, and 2) out-of-phase or alternate movements (Viala et al., 1986). After the training period, rabbits subjected to the in-phase training showed a synchronous hindlimb pattern for locomotion, those who experienced out-of-phase training showed exclusively alternate stepping of the hindlimbs, while those who received both forms of training showed both hindlimb coordination patterns for locomotion (Viala et al., 1986). Like spinal animals, use-dependent plasticity at the subcortical level has been demonstrated in human infants (Yang et al., 1998; Zelazo et al., 1972), and our findings in chapter 3 also support this idea. In addition, we show that the effects of training one type of interlimb coordination in weight-bearing may translate to non-weight-bearing activities, such as kicking in supine.

1.12 References

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Chapter 2: Phase-Cycle Relationship of Rhythmic Movements in Human Infants is a Function of Load

2.1 Introduction

Rhythmic locomotor movements produced by legged animals typically consist of two phases. There is a powerstroke when the animal exerts force with its limb against a substrate for propulsion and a returnstroke when a much smaller force is exerted to return the limb in preparation for the next powerstroke. The powerstroke of walking is the stance or extension phase, while the returnstroke is the swing or flexion phase. Behavioral studies of walking have shown that the extension phase varies with the cycle duration while the flexion phase varies much less if at all (Grillner et al., 1979; Halbertsma, 1983; reviewed in Rossignol 1996). We will refer to this phenomenon as an extension asymmetry. This asymmetry in the control of extension and flexion phases has been reported for virtually every terrestrial animal studied (Arshavskii et al., 1965; Delcomyn, 1971; Goslow et al., 1973; Grillner et al., 1979; Williams, 1981; Jacobson and Hollyday, 1982; Vilensky, 1983; Reilly and Delancey, 1997; Leblond et al., 2003; reviewed in Grillner, 1981; Hildebrand, 1985), and indeed is often used as a hallmark of locomotor behavior.

Rhythmic locomotor activity can be generated by a group of neurons in isolation, called the central pattern generator (CPG). Occasionally in preparations in which afferent input is small or absent, the flexion phase has been reported to vary strongly with cycle duration (referred to as a flexion asymmetry) (Grillner and Zangger, 1979; Bekoff et al., 1987; Douglas et al., 1993), or both phases have been found to vary to the same extent

with cycle length (Baker et al., 1984; Kiehn and Kjaerulff, 1996). Recently Yakovenko et al. (2005) studied fictive locomotion in adult decerebrate cats with stimulation to the midbrain locomotor region (MLR). They found variations in cycle length to be more strongly related to changes in flexor duration than extensor duration in 70% of preparations. Hence, the evidence is not strong for the isolated CPG to have an inherent asymmetry in the control of extension and flexion phases.

Is the behavioral phenomenon of extension asymmetry, seen in normally behaving animals, the result of other factors such as sensory input generated by the powerstroke? Movement-related afferent input modulates the CPG output to meet environmental and task demands (reviewed in Pearson, 1993; Grillner et al., 1997; McCrea 2001; Delcomyn, 2004). Numerous studies involving intact, decerebrate, spinal and fictive preparations have demonstrated that input from the extensor muscle afferents that detect stretch and load during the extension phase prolongs ipsilateral extension and inhibits the transition to flexion (Duysens and Pearson, 1980; Conway et al., 1987; Pearson et al., 1992; Guertin et al., 1995; Whelan et al., 1995; Pang and Yang, 2000; reviewed in Whelan, 1996; McCrea, 2001). Similarly, activation of group I afferents of hindlimb flexors prolongs ipsilateral flexion in decerebrate and fictive preparations (Perreault et al., 1995; Hiebert et al., 1996; Quevedo et al., 2000; Lam and Pearson, 2001). It may be that the isolated CPG does not have an asymmetry in the control of extension and flexion phases, but instead one phase is preferentially modulated depending on the nature of the afferent input the CPG receives. Extension asymmetry, reported for terrestrial locomotion, may be a result of gravity imposing greater load and afferent feedback on the extension phase.

To explore this idea, we examined how loading either the flexion or extension phases affects the relationship between phase durations (flexion and extension) and cycle duration for stepping-like movements in human infants.

Humans under the age of one year are an excellent model for the study of brainstem/spinal-mediated locomotion (Yang et al., 1998, 2004) since cortical control of leg movements is limited at this age (Peiper, 1961; Yakovlev and Lecours, 1967; Brody et al., 1987; Eyre et al., 1991; Khater-Boidin and Duron, 1991; Szelenyi et al., 2003). We hypothesized that during rhythmic movements the phase experiencing greater load (i.e., powerstroke) will be the phase whose duration varies more strongly with cycle duration. The results supported our hypothesis. Hence, the asymmetry in extension and flexion phases seen during rhythmic leg movements is the result of sensory input and does not reflect an inherent characteristic of the locomotor CPG.

2.2 Materials and Methods

Subjects

Fifty-nine healthy infants aged 3 – 10 months were recruited through parent/infant groups at public health clinics in Edmonton. Infants and their parents attended one testing session lasting about one hour. Informed consent was obtained from a parent at the time of testing. Ethical approval was obtained from the Health Research Ethics Board of the University of Alberta and Capital Health, Edmonton.

Experimental Procedures

Six rhythmic movements were studied, in which 3 provided resistance against extension (load-extension condition), 2 provided resistance against flexion (load-flexion condition), and 1 provided similar resistance to both flexion and extension (neutral condition). Rhythmic leg movements in infants are more easily elicited when arousal level is high (Thelen, 1985); therefore, a researcher and/or parent attempted to excite the infant through play. One or two trials (each lasting 1 – 2 minutes) of each rhythmic movement were attempted. Short rests of 1 – 2 minutes were taken between trials. The infant's body weight was measured at the end of the experiment.

Neutral condition:

Kicking in supine. The resistance experienced during the flexion and extension phases was about equal when kicking in the supine position since the movements for both phases occurred mainly in the transverse plane, where gravitational resistance to leg movement is considerably reduced compared to an upright posture (Jensen et al., 1994). Infants kicked while lying supine on a play mat.

Load-extension conditions:

1. Stepping on a treadmill. The resistance experienced during the extension/stance phase of stepping, when force was exerted against the treadmill belt, was far greater than the resistance experienced during the flexion/swing phase, when gravity resisted lifting of the leg. This can be seen from the magnitude of muscle moments generated in the lower limb during adult walking (Winter, 1983). A researcher held the infant

under the arms over a treadmill, allowing the infant to support as much of his/her weight as possible without the infant's knees collapsing into flexion. The researcher's forearms were supported by a platform to minimize imposing movements on the infant. We found that older infants tended to support a greater percentage of their body weight than younger infants. To rule out the possibility that the extension asymmetry seen in stepping becomes greater with age rather than by the amount of load experienced by the legs, 3 older infants stepped on the treadmill while the amount of weight-bearing was manipulated by the researcher holding the infant. These infants stepped under 2 conditions: 1) normal weight support, in which the infants were encouraged to support as much of their body weight as possible, with support provided only as needed for safety, and 2) low weight support, in which the researcher provided consistent force upwards, to support more of the infant's body weight. The manipulations were applied for 20-second periods while the infants were stepping.

2. Kicking against a surface. The infants kicked in a supine position with their feet contacting a firm surface during the extension phase. For this rhythmic movement, resistance was greatest during extension when the feet exerted force against the surface. The firm surface against which the infants kicked was a hard piece of Styrofoam stabilized by a researcher (3 infants) or a Plexiglas plate instrumented with a force transducer (5 infants) (see Instrumentation).
3. Kicking in sitting. The infants were seated at the edge of a small bench allowing their legs to dangle over the edge. Kicking in this position occurred predominantly at the

knee joint, with little hip movement. Resistance was encountered as the knees extended against gravity, while gravity assisted the flexion phase.

Load-flexion conditions:

1. Air-stepping. Air-stepping is a stepping-like movement elicited when infants are held upright in the air. The infants were held under the arms without their feet touching a surface. Gravity provided resistance during the flexion phase in this position, while it assisted the extension motion. The resistance to hip flexion movement as a result of gravity is 4 times greater in an upright kicking posture compared to the supine position (Jensen et al., 1994).
2. Weighted air-stepping. Air-stepping was also attempted with the infants wearing 0.5 lbs or 1.0 lbs weights on each ankle, thus providing additional resistance to the flexion phase than air-stepping alone. The extra load of the ankle weight was calculated to vary from 24% to 56% of the weight of the infants' legs. To estimate this extra load, the ankle weight worn by an infant was expressed as a percentage of his/her leg weight. Leg weight was estimated as 10.9% of body weight (Schneider et al., 1990).

Instrumentation

Twin-axis electrogoniometers (Penny and Giles, Biometrics Ltd., Blackwood Gwent, UK) were placed over the right and left knee joints. The knees were chosen rather than the hips since preliminary data showed knee flexion/extension to be characteristic of all 6 rhythmic movements, in contrast to hip flexion/extension, which was minimal when the

infants kicked in sitting. The arms of the goniometers were aligned with the long axes of the femur and tibia.

All trials were videotaped (30 frames/second, Canon Elura 50). The infants were videotaped in the sagittal plane, with the exception of the trials where the infants were kicking against the kick plate. For these trials the camera was placed at an angle to the sagittal plane due to space constraints in the lab. The leg fully visible in the video was the reference leg. White adhesive markers were applied to the reference leg in the following locations: midline of the trunk above the iliac crest, greater trochanter, lateral knee joint line, lateral malleolus and head of the fifth metatarsal. Markers were also applied to the medial knee joint line, medial malleolus and medial aspect of the great toe of the contralateral leg.

Surface electromyographic (EMG) recordings were obtained from the quadriceps and hamstrings bilaterally for all infants, and from the gastrocnemius-soleus or tibialis anterior bilaterally in some infants. EMG recordings from the gastrocnemius-soleus and tibialis anterior were not collected from all infants since preliminary data showed that these were not consistently phasic muscles for the rhythmic movements studied. Two miniature electrodes with a 2 mm recording diameter (Kendall, Chicopee, MA) were placed 1cm apart on the above-mentioned muscles. Black leotards covered the electrodes and corresponding wires.

A split-belt treadmill was used in this study because it is equipped with separate force plates for the right and left legs, allowing accurate measurement of vertical forces for each limb (model INFSBT-FP, designed and built by R Gramlich and S Graziano, University of Alberta, Edmonton, AB) (Yang et al., 2005). A Plexiglas partition between the right and left treadmill belts helped to keep the legs on separate belts. The 2 belts were always run at the same speed, between 0.1 – 0.6 m/s depending on the infant's ability.

The Plexiglas kick plate (2 cm thick, 26 cm high, 34.5 cm wide) was attached to an instrumented uniaxial force transducer (model 60001A50-1000, Intertechnology, Inc, Toronto, ON) that was securely mounted to a wall. Prior to its use, the custom-made plate was tested to ensure accurate readings regardless of the position on the plate with which force is exerted.

A custom made digital counter synchronized the video and analog signals at a rate of 1 Hz. The EMG signals were high-pass filtered at 10 Hz and rectified. All signals (rectified EMG, goniometer, force plate and kick plate) were low-pass filtered at 30 Hz and converted from analog to digital form at 250 Hz (Axoscope, Axon Instruments Inc., Foster City, CA). The raw signals were recorded on video tape for back-up (A.R. Vetter, Redersburg, PA).

Analysis

The video recordings were reviewed to identify sequences of rhythmic movements. An infant's data were included for analysis if the following criteria were met: 1) the infant performed sequences of leg movements, defined as at least 2 sequential knee flexion-extension movements in both legs (with no pauses longer than 1 second and change in knee angle $\geq$20 degrees in both directions), in at least 1 of 6 rhythmic movements, and 2) the combined total number of flexion-extension cycles from these sequences was 10 or more for any 1 rhythmic movement.

Flexion and extension phase durations and cycle durations, measured from the knee goniometer signals, were determined for every sequence of movement using a customized software program (Matlab, MathWorks, Inc., Natick, MA). Cycle duration was defined as the onset of knee flexion to the onset of the subsequent knee flexion. The flexion phase was defined as the onset of knee flexion to the onset of knee extension, and the extension phase was considered the onset of knee extension to the onset of flexion (Figure 2.1, solid and dotted vertical lines show onset of flexion and extension, respectively, for 1 movement). Average values for the durations of the cycle, flexion phase and extension phase were calculated for each infant.

Some infants performed rhythmic leg movements in an alternate manner (i.e., one leg flexes while the other extends), while others preferred synchronous movements. In the weight-bearing position, synchronous movements resembled hopping on the treadmill. In order to be considered hopping, the synchronous movement must have involved both

legs. A customized software program (Matlab, MathWorks, Inc., Natick, MA) was used to determine the phase lag between the 2 legs. To calculate the phase lag, the onset of a cycle in the contralateral limb was expressed as a percentage of the cycle of the reference limb. A synchronous movement was defined as one in which the phase lag was 0 - 20% or 80 - 100%, whereas an alternate movement was characterized by a phase lag of 20.1 – 79.9%. Both interlimb forms of the rhythmic movements were included for analysis.

For each of the 6 rhythmic movements, a customized software program (Matlab, MathWorks, Inc., Natick, MA) was used to determine average quadriceps, hamstring, gastrocnemius-soleus and tibialis anterior burst durations and amplitudes for each infant. EMG data from an infant was included for further analysis only if there were at least 10 clear bursts/muscle, for at least 1 of the 4 muscles, and there was no evidence of crosstalk between antagonist muscles (see Statistics). EMG duration was defined as the time from the onset to the offset of EMG bursting. Onset and offset of EMG was defined as activity that crossed a pre-selected threshold value, which varied between 0.2 and 0.5 v. EMG amplitude was calculated by averaging EMG activity within each burst. For each infant, the average EMG duration and amplitude obtained for each muscle during each rhythmic movement was normalized to that infant's average duration and amplitude values for kicking in supine (assigned a value of 100%), as most infants provided good EMG data for kicking in supine. The peak force (in Newtons) exerted by the infants during the extension phase of every step and kick against the kick plate was measured and the average peak force across cycles was expressed as a percentage of the infant's body

weight. If an infant kicked synchronously against the kick plate, the peak force of each kick of the reference leg was estimated by dividing the observed peak force in half.

Statistics

A one-way analysis of variance was used to compare the duration of cycle and phase across rhythmic movements (Bonferroni correction used for post-hoc analyses). Independent t-tests were used to compare cycle durations of the alternate and synchronous forms of a rhythmic movement. Linear regression analyses were performed to determine the relationship between flexion/extension phase duration and cycle duration (we will refer to this as the phase-cycle relationship) for each rhythmic movement. The significance of the regression of flexion/extension phase on cycle duration was assessed with an F-test. To compare the slopes of the regression lines for the flexion and extension phases within each movement, a paired t-test was used. Welch's t-test, appropriate for comparing data sets differing in sample size or variability (Glass and Hopkins, 1996), was used to compare regression line slopes of alternate and synchronous forms of a movement and also across movements. Pearson's Product-Moment correlation coefficients were used to determine if there was crosstalk between recordings of antagonist muscles. If the correlation coefficient exceeded 0.3 for any pairs of EMG recordings, those recordings were not included for analysis. Paired t-tests were used to compare EMG burst durations and amplitudes across rhythmic movements. Significance was set at $p<0.01$ for all statistical tests due to the large number of tests performed. All mean values reported are accompanied by one standard deviation.

2.3 Results

Of the 59 infants tested, 40 (aged 3.2 – 10.1 months) provided useful data for at least one rhythmic movement. For each rhythmic movement studied, details of the number of infants and the number of cycles are shown in Table 2.1.

Description of Rhythmic Movements

All 6 rhythmic movements consisted of alternating phases of knee flexion and extension. Figure 2.1 shows raw data from the goniometer of the reference knee during each of the movements from individual subjects. It is clear that there are differences in cycle and phase durations between movements. The average cycle durations of stepping and kicking against a surface were longer than the average cycle durations of the other movements (all comparisons $p<0.001$, except $p = 0.129$ for kicking against a surface versus kicking in supine) (Figure 2.1). The average cycle duration of stepping was significantly longer than that of kicking against a surface. Stepping and kicking against a surface were also characterized by longer extension phases ($p<0.001$), which typically consisted of rapid extension followed by a slower extension period or a plateau in knee extension prior to the onset of flexion. This was sometimes observed in kicking in sitting as well.

Comparison of Alternate and Synchronous Forms

The alternate and synchronous forms of rhythmic movements were first compared to determine whether it was appropriate to combine the 2 types of interlimb coordination for analysis. Since our primary interest was the phase-cycle relationship, we compared this

relationship for synchronous and alternate forms of the movements. No differences were found between the phase-cycle relationship for alternate and synchronous forms of the rhythmic movements (Figure 2.2, comparison of kicking against a surface was performed but not shown since results similar to other 4 movements). A comparison was not performed for weighted air-stepping due to the small number of infants who showed this behavior (i.e., only 1 showed alternate coordination and 3 showed synchronous coordination). Considerable overlap of data points for alternate and synchronous forms of the movements was seen for both the extension and flexion phases (Figure 2.2). The cycle durations of the alternate forms of the movements were found to be longer than the synchronous forms ($p<0.01$, except $p = 0.02$ for air-stepping). In spite of the differences in cycle duration, the phase-cycle relationship was identical for alternate and synchronous forms of a movement. For that reason, we felt justified to pool the data for comparison of phase-cycle relationship.

Phase-Cycle Relationships

All linear regressions performed were statistically significant. Both extension and flexion phase durations varied with cycle duration for kicking in supine. Identical slopes and r^2 values were found for the regression lines describing the relationships of cycle duration with flexion and extension phase durations (Figure 2.3a). Movements of the load-extension condition showed an extension asymmetry (Figure 2.3b). Slopes of the phase-cycle regression lines for extension were found to be statistically greater than those for the flexion phase. The r^2 values were greater for the extension phase, indicating that the relationship between phase duration and cycle duration was stronger for extension than

flexion. Air-stepping and weighted air-stepping (load-flexion condition) showed a flexion asymmetry (Figure 2.3c). The slopes of the regression lines for the flexion phase were significantly greater than those of the extension phase. The r^2 values were also high for the flexion phase-cycle relationship.

Amount of Load Affects Phase-Cycle Relationship

To further examine the relationship between the degree of load and the phase-cycle relationship, the data from stepping and kicking against a surface were rearranged as follows. The average peak force exerted by the infants during stepping/hopping on the treadmill ranged from 17.9 – 46.9% of their body weight. So, the phase-cycle relationship was recalculated for the 3 infants who stepped with the lowest average forces of the group (17.9 – 22.3%, low force group) and the 3 infants that showed the greatest average normal forces (38.4 – 46.9%, high force group) (Figure 2.4a). The low force group showed less of an extension asymmetry than the high force group, although the slopes of the regression lines of the extension phase were not significantly different from one another. The average ages of the high and low force groups were 9.0 $\pm$ 1.2 and 5.1 $\pm$ 1.3 months, respectively. To rule out age as a potential factor for the difference in the phase-cycle relationships, 3 older infants (mean age = 9.5 $\pm$ 1.0 months) were tested while stepping on the treadmill under 2 load conditions: stepping with normal weight support (averaged 62.0 $\pm$ 6.5% body weight) and stepping with low weight support (averaged 26.6 $\pm$ 4.5% body weight). There was a considerable change in the phase-cycle relationship under the 2 stepping conditions ($p>0.01$) (Figure 2.4b). Again, the strength of the extension asymmetry was stronger with the higher load.

A similar relation was seen in kicking. The force exerted against the kick plate ranged from 13.7 – 46.7% of body weight. The extension asymmetry was slightly greater in infants who exerted more than 20% of their body weight (n = 3, mean force = 31.4 $\pm$ 13.6%) than those who kicked with a force equal to or less than 20% of their body weight ($p>0.01$) (n = 2, mean force = 15.8 $\pm$ 3.0%) (Figure 2.4c). Hence, load seems to determine the strength of the phase-cycle relationship.

EMG Recordings

Acceptable EMG recordings were obtained from the quadriceps, hamstrings and gastrocnemius-soleus muscles. All 6 movements were characterized by alternate bursting of the quadriceps and hamstrings; the former bursting during the extension phase and the latter during the flexion phase. The gastrocnemius-soleus muscles tended to burst along with the quadriceps during the extension phase of the movements. The patterns observed in the phase-cycle relationships were mirrored in the muscle activity. Mean quadriceps and hamstrings burst durations were found to vary according to movement condition (Figure 2.5). There was a trend for movements with higher load during extension to show longer quadriceps bursts and shorter hamstrings bursts relative to kicking in supine. Conversely, air-stepping, which loads the flexion phase, had a shorter quadriceps burst and a longer hamstrings burst compared to kicking in supine. However, only the quadriceps burst duration was significantly different between kicking against a surface and kicking in supine (Figure 2.5a). No trends were observed in the burst durations of the gastrocnemius-soleus muscle across movements. The variability in burst durations

was high and the number of infants included in EMG analysis was small. No trends were found in the EMG amplitudes across movement conditions.

2.4 Discussion

This study demonstrates that stepping-like movements in human infants are not all characterized by an extension asymmetry. The phase experiencing the greater load-related afferent feedback is the phase that lengthens and varies with cycle duration. It appears this general principle is true for a variety of rhythmic leg movements across vertebrate species.

Methodological Issues

First, we were unable to directly measure the load experienced by the muscles. Instead indirect measures of vertical ground reaction and horizontal normal forces were used to estimate the load of the extensor muscles. Load on the flexor muscles was also indirectly estimated by the limb load (Schneider et al., 1990) and the extra weights. The load experienced during kicking in supine was estimated from the literature (Jensen et al., 1994). Nonetheless, in each condition we were able to manipulate the load sufficiently to induce a clear difference in loading of flexion and extension phases, allowing us to test our hypothesis.

Secondly, we were unable to record from the important hip flexor muscles because they are not accessible from the surface. Moreover, good EMG data is difficult to obtain from human infants due to fat tissue overlying the muscles and the high possibility of crosstalk

between muscles. Hence, we relied mostly on kinematic data to define flexion and extension phases. This must be considered when interpreting our findings since kinematic data does not directly represent CPG output.

Thirdly, we assumed that all the rhythmic movements represented outputs from the same CPG, but we cannot confirm this unequivocally. The following, however, provide supporting evidence for shared circuitry. 1) We observed kinematic similarities in the movements. All three behaviors involved rhythmic alternation between phases of knee flexion and extension. 2) We have observed infants to make smooth, continual transitions between two different behaviors, such as air-stepping and stepping on a treadmill. These transitions are characterized by no breaks in the movement (i.e., no pauses longer than 0.5 seconds) and no significant changes in cycle duration throughout the transition. Others have reported similar kicking behavior in different postures (supine and upright) (Jensen et al., 1994). Smooth transitions have been observed in other animal preparations in which more definitive evidence for shared circuitry has been obtained (reviewed in Stein 2005). 3) The primary differences we observed between the movements are the timing of the cycle and phases, which can be explained by our hypothesis. In other words, the response to sensory input associated with load was the same across various types of rhythmic movements. The fact that load alters the timing of the CPG in the same way strongly suggests the behaviors share some common circuitry.

Phase-cycle Relationship is a Function of Load

The findings presented in this study show that altering load, either through minor changes in the task or changes in the degree of load experienced in the same task, affects the phase-cycle relationship. We showed that load feedback has access to the pattern generator regardless of the phase it is applied. Previous work in animals (Conway et al., 1987; Hiebert et al., 1996; reviewed in McCrea, 2001), human infants (Pang and Yang, 2000) and adults (Stephens and Yang, 1999) showed that the strength and duration of muscle activation during the stance phase of walking are affected by load. More recently, similar findings were reported for the swing phase (Lam and Pearson, 2001). The natural extension of this response to load on the phase-cycle relationship, however, had not been addressed previously.

Examples of this phenomenon have been reported in the literature, supporting this unifying explanation for the phase-cycle relationship. Reports of air-stepping in chicks (Johnston and Bekoff, 1992, 1996), spinal kittens (Bradley and Smith, 1988) and monkeys (Vilensky et al., 1989) have suggested a flexion asymmetry. In swimming, cycle duration was equally divided between extensor and flexor muscles in chicks (Johnston and Bekoff, 1992, 1996) and turtles (Earhart and Stein, 2000). Since the load experienced by the limbs during swimming depends on the speed of movement, it is possible that the flexion and extension phases demonstrate a variety of relationships depending on the speed of the movement in each phase. Terrestrial locomotion shows an extension asymmetry because typically animals walk upright, resulting in gravity being dominant for the extension phase.

The effect of load on the phase-cycle relationship was most directly stated by Bekoff and colleagues (Bekoff et al., 1987; Johnston and Bekoff 1992, 1996). By comparing walking, swimming, air-stepping and hatching in the chick, they suggested that the phase-cycle relationship may depend on sensory input. This suggestion is supported by their early work in which deafferentation of the limbs in chicks resulted in walking movements that did not show an asymmetry in the control of flexion and extension phases (Bekoff et al., 1987).

Exceptions to our hypothesis are largely explainable. Thelen and colleagues (1981) reported phase-cycle relationships during infant kicking in supine to have an extension asymmetry. They defined the swing phase of kicking as the time from onset of flexion of the leg to the end of the subsequent extension of the leg. The stance phase was defined as the pause in leg movement between successive kicks, which varied in duration from about 1 to 9 seconds (Thelen et al., 1981). It is not surprising that they found the stance phase of kicking to vary strongly with cycle duration since the inclusion of such long pauses must have dominated the cycle duration. Earhart and Stein (2000) found slopes of the phase-cycle relationship to be the same for flexion and extension phases of forward swim and backpaddle behaviors of the turtle. They defined the powerstroke of forward swim to occur during hip extension and that of the backpaddle swim to occur during hip flexion (reviewed in Stein, 2005). There was no quantification of the load, however, so it remains unclear how different the loads were between the flexion and extension phases. Load during swimming is largely viscous, and therefore, dependent on the velocity of the strokes. If the velocity of the movement is similar for the returnstoke and powerstroke

phases of swimming, then the loads might also be similar. For scratch behaviors of the turtle, the powerstroke was defined as the phase when the limb rubs against the body (reviewed in Stein, 2005). By this definition, the powerstroke is hip flexion for rostral scratch and hip extension for pocket and caudal scratches (reviewed in Stein, 2005). As we would expect, caudal scratch showed an extension asymmetry (Earhart and Stein, 2000). However, in contrast to our hypothesis, both extension and flexion phase durations varied with cycle length for rostral scratch, and pocket scratch showed a slight flexion asymmetry (Earhart and Stein, 2000). The loads experienced by the muscles during each phase of these scratch movements have not been quantified. Indeed, the definition of powerstroke and returnstroke was based on contact with a substrate. Thus, future work to quantify the load might be helpful to determine if our hypothesis holds true for swimming and scratching in turtles.

Afferent feedback other than load may also influence phase-cycle relationships. The relationship between duration of activity of a flexor muscle (tibialis anterior) and the cycle duration during air-stepping was made steeper when tape was applied to the paw of a spinal cat (Giuliani and Smith, 1985). Thus, it is possible that other afferent input can affect the phase-cycle relationship. Alternatively, the stronger contraction of the flexor muscles induced by the application of tape could lead to greater loading of those muscles, which would influence the phase-cycle relationship.

Concluding Remarks

Locomotion has traditionally been thought to have an extension asymmetry since most reduced and intact preparations have shown the extensor phase to vary with cycle duration, in contrast to the flexor phase which remains relatively invariant (Griller and Zangger, 1979; Griller and Dubuc, 1988; Pearson and Rossignol, 1991; reviewed in Griller, 1981; Rossignol, 1996). We showed that there is not an inherent asymmetry in the control of extension and flexion for locomotion. The greater the load experienced by either the flexion or extension phase of a stepping-like movement, the longer that phase and the greater the asymmetry. The CPG for locomotion can modulate the duration of either phase based on the afferent input it receives.

Condition	Movement	Number of Infants	Number of Cycles
Load-extension	Stepping/Hopping	25	797
	Kicking Against a Surface	8	131
	Kicking in Sitting	5	141
	Stepping (Normal Weight Support)	3	64
	Stepping (Low Weight Support)	3	59
Load-flexion	Air-stepping	11	196
	Weighted Air-stepping	4	67
Neutral	Kicking in Supine	27	669

Table 2.1: The number of infants and the number of cycles included for analysis for each rhythmic movement. Seven of the 8 infants who kicked against a surface also kicked in supine. All 4 of the infants who air-stepped with weights also air-stepped without weights.

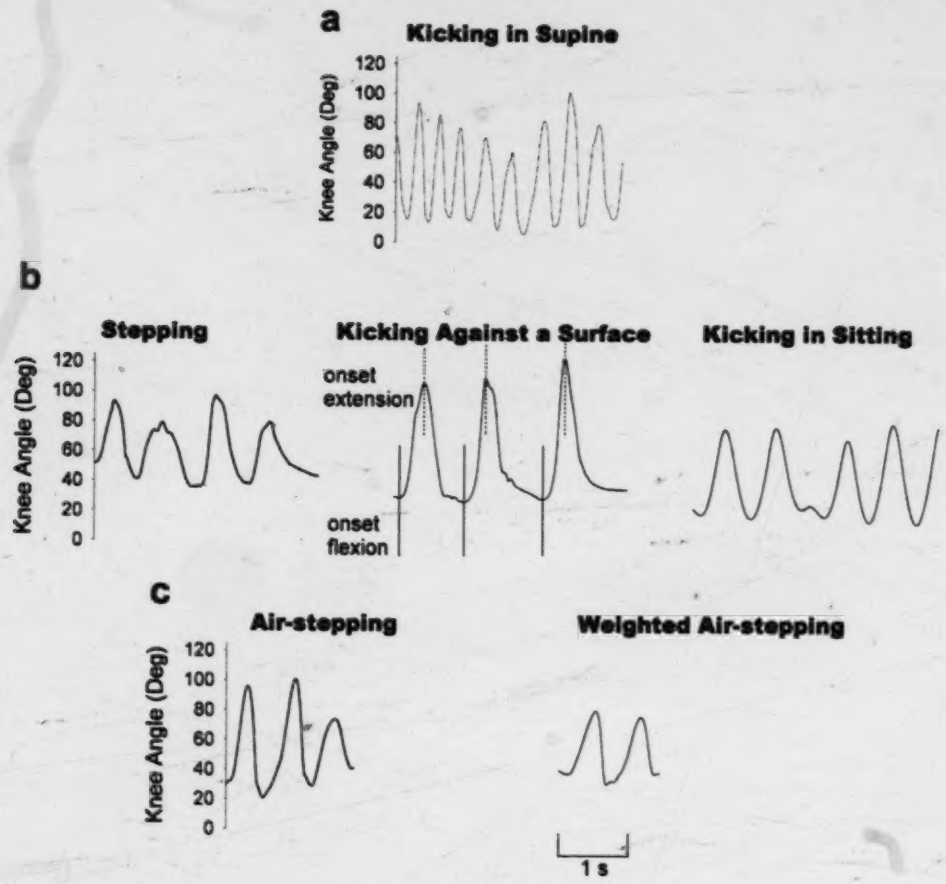

Figure 2.1: Movement of the knee in the 6 rhythmic behaviors. Goniometer signals of the reference knee from single infants for neutral condition (a), load-extension condition (b) and load-flexion condition (c). Air-stepping and weighted air-stepping traces are from the same infant, all other traces are from different infants. Increasing angle indicates flexion. Solid and dotted vertical lines mark onset of flexion and extension phase, respectively, shown for just one condition (kicking against a surface).

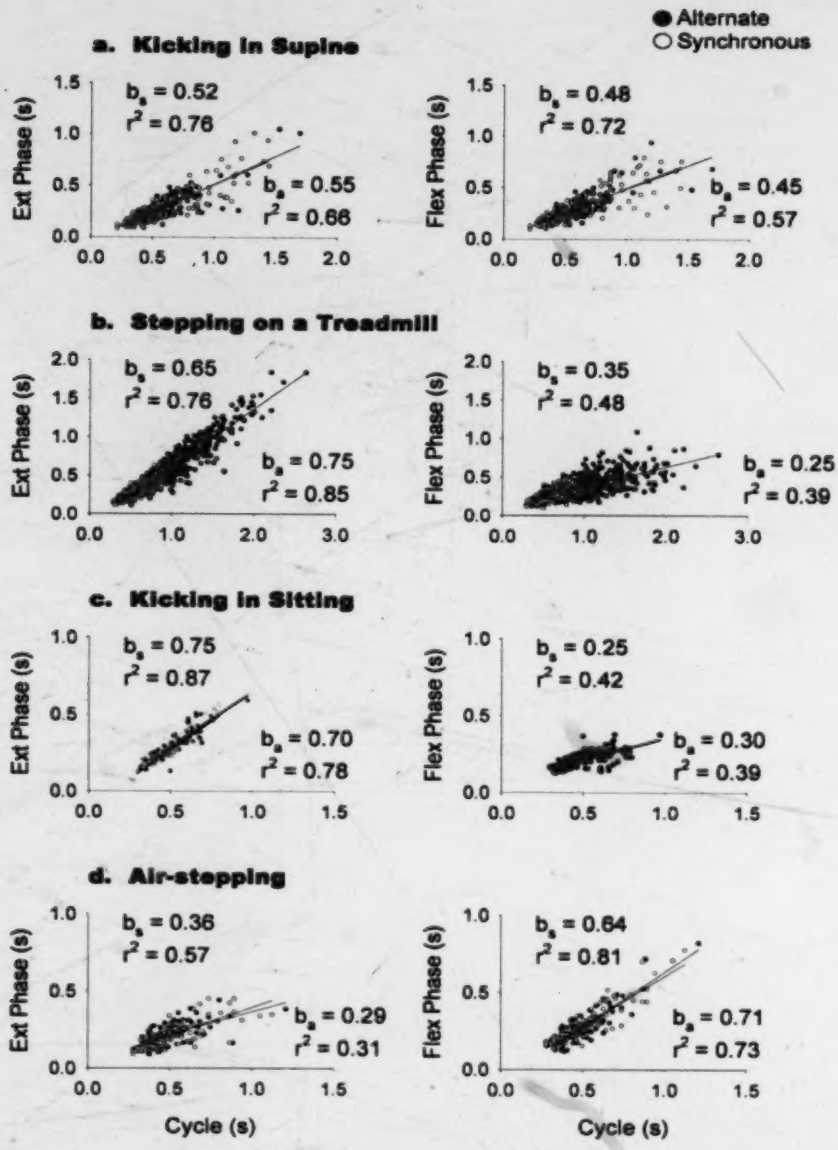

Figure 2.2: Comparison of alternate and synchronous forms of 4 movements (kicking in supine (a), stepping (b), kicking in sitting (c) and air-stepping (d)). Relationships between phase and cycle duration are shown for the extension (left panel) and flexion (right panel) phases. Each dot represents 1 cycle from 1 infant. The best fitting straight line was estimated for alternate and synchronous forms of the movements separately. Slopes of the regression lines describing alternate and synchronous data are represented by b_a and b_s, respectively. No differences were found between slopes of alternate and synchronous forms for flexion and extension phases of each movement ($p>0.01$).

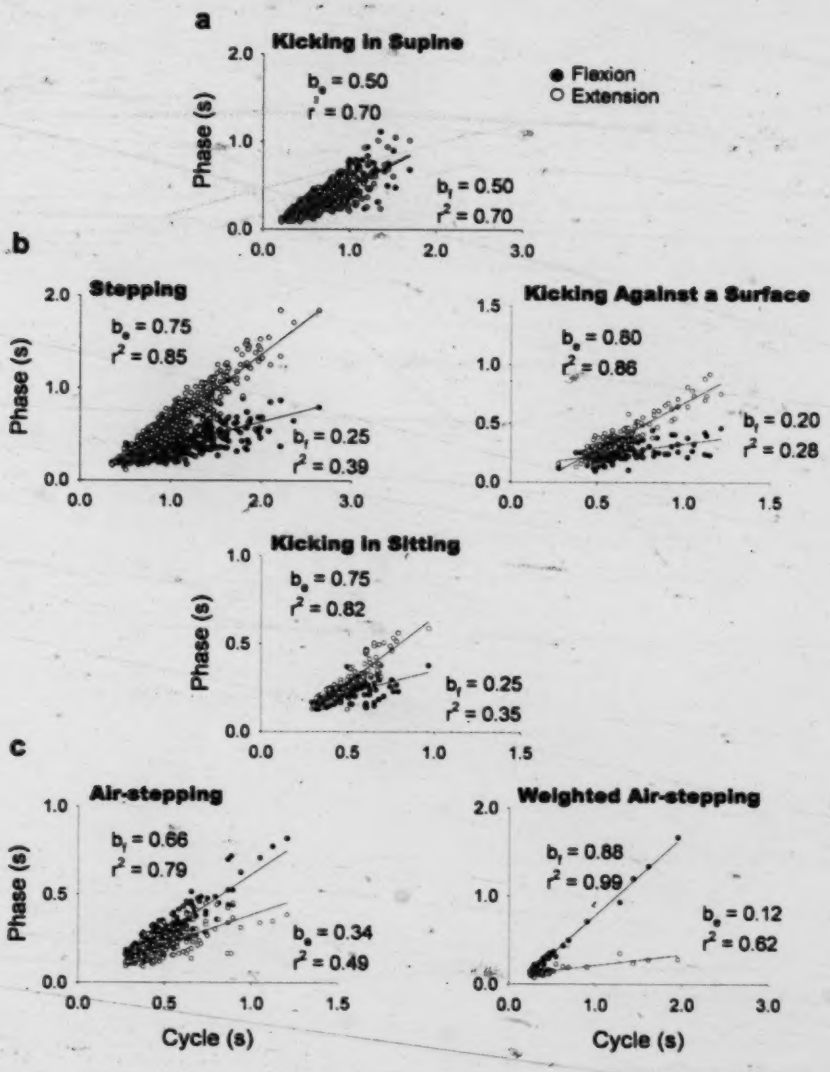

Figure 2.3: Phase-cycle relationships for neutral condition (a), load-extension condition (b) and load-flexion condition (c). Each dot represents 1 cycle from 1 infant. The best fitting straight line was estimated for extension and flexion phases of each movement separately. Slopes for the flexion and extension phases are represented by b_f and b_e, respectively. The slopes for flexion and extension phases were the same for kicking in supine (a). The slope for extension was significantly greater than that for flexion when load was applied during extension (b, $p<0.01$). The reverse was true for loads applied in flexion (c, $p<0.01$).

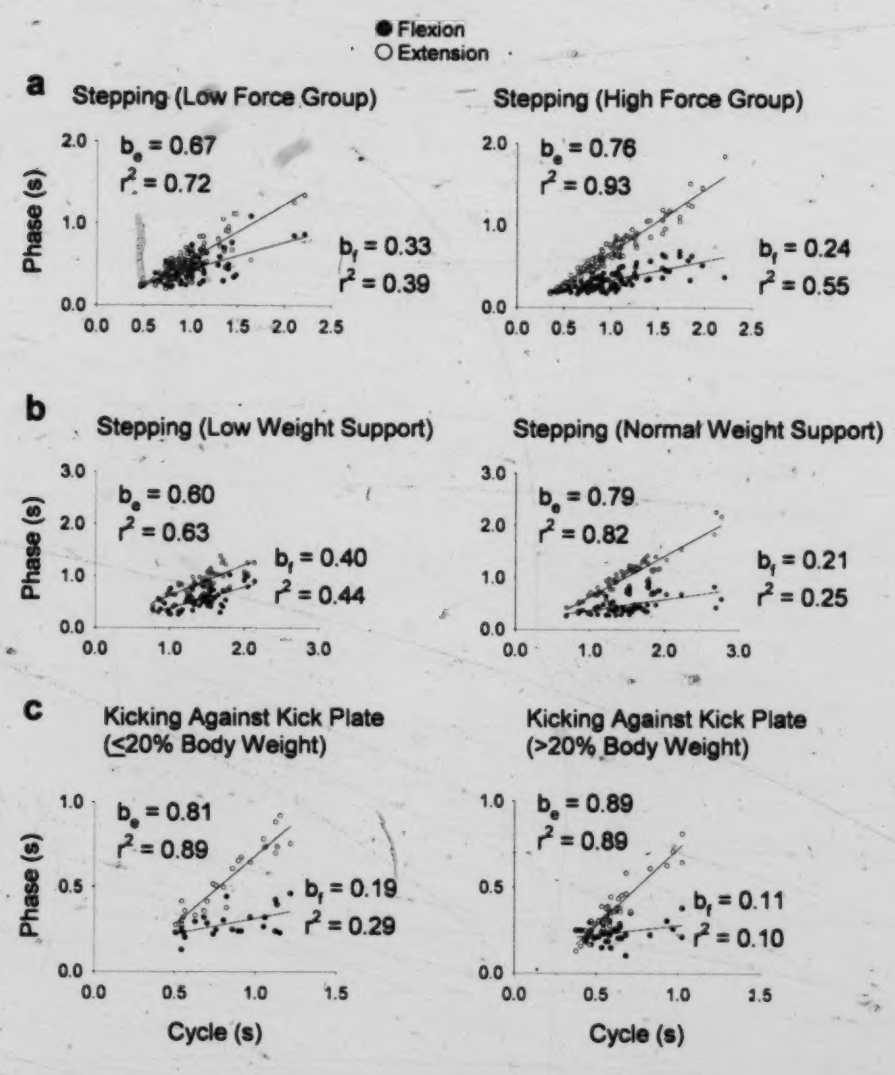

Figure 2.4: Comparison of the phase-cycle relationship when loads were different in the same movement condition. (a) Data from infants who showed low (left) and high (right) weight support during treadmill stepping. (b) Data from 3 infants in stepping when the researcher manipulated the load. (c) Data from infants who showed low (left) and high (right) loads when kicking against a kick plate. All conventions in graphs are the same as Figure 2.3.

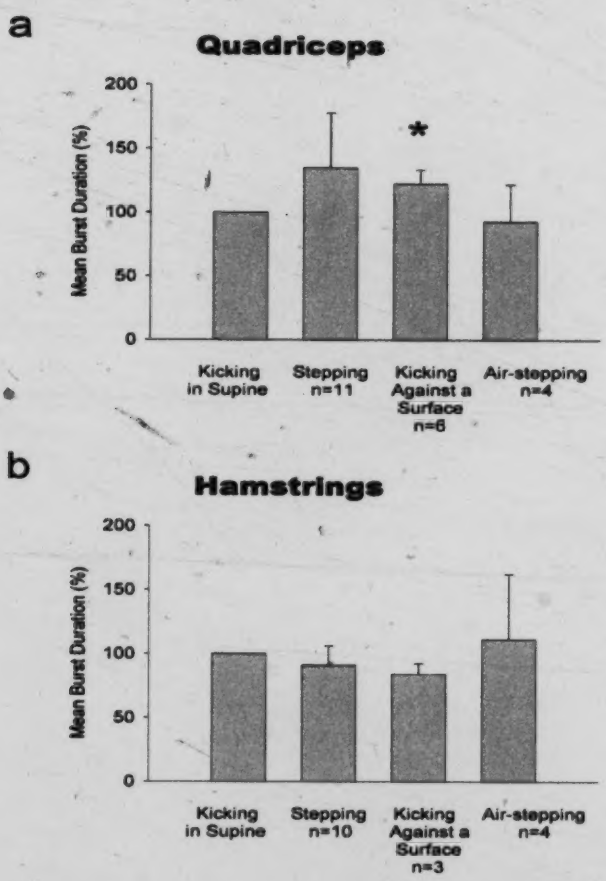

Figure 2.5: Mean burst durations of the quadriceps (a) and hamstrings (b) compared across movements. Mean burst durations are expressed as a percentage of the mean burst duration for kicking in supine. * indicates a significant change in duration compared to kicking in supine. Error bars represent 1 standard deviation.

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Chapter 3: Interlimb Coordination of Rhythmic Movements in Human Infants and the Role of Motor Experience

3.1 Introduction

Animals, including humans, are capable of many forms of rhythmic leg movements, such as kicking, cycling, swimming, walking and hopping. It is thought that the neuronal circuitries underlying different rhythmic movements are not separate entities, but rather networks that share common circuitry (reviewed in Marder and Calabrese, 1996; Dickinson et al., 2000; Kupfermann and Weiss, 2001). Do the rhythmic movements performed by humans share underlying circuitry? One way to address this question is to look at how the limbs are coordinated in different rhythmic movements to see if similar patterns are expressed.

During rhythmic movements, the homologous limbs on each side of the body are typically coordinated in one of two ways: 1) an out-of-phase or alternate interlimb coordination (i.e., locomotor cycle of one limb begins when the other limb has completed 50% of its cycle), or 2) an in-phase or synchronous interlimb coordination (i.e., onsets of locomotor cycles for both limbs are simultaneous). Expression of the two types of interlimb coordination is likely made possible by using different neural circuitry connecting the pattern-generating spinal networks of the two limbs (Forssberg et al., 1980; Halbertsma, 1983). Recent work in rodents has increased our understanding of the spinal circuitry governing interlimb coordination in vertebrates (Kremer and Lev-Tov, 1997; Butt and Kiehn, 2003; reviewed in Kiehn and Butt, 2003). The production of alternate hindlimb movements is thought to involve commissural glycinergic inhibitory

pathways, while commissural glutaminergic excitatory pathways regulate the synchronous pattern (Kremer and Lev-Tov, 1997; reviewed in Kiehn and Butt, 2003). Preferential expression of the alternate pattern for walking may result from the inhibitory pathways being stronger than the excitatory pathways (reviewed in Kiehn and Butt, 2003).

Human infants can express both types of coordination when kicking and when stepping on a treadmill. At this young age, these leg movements are under limited input from the brain (Peiper, 1961; Yakovlev and Lecours, 1967; Brody et al., 1987; Eyre et al., 1991; Khater-Boidin and Duron, 1991; Szelenyi et al., 2003). We wondered whether the circuitry responsible for infant stepping is also involved in infant kicking. To address this idea, we asked two questions. First, does an infant exhibit the same interlimb coordination during these different rhythmic movements? Secondly, can practice of one type of interlimb coordination in a weight-bearing position (i.e., stepping or hopping) translate into a change of the preferred coordination for a non-weight-bearing rhythmic movement (i.e., kicking)?

If infant kicking and stepping share underlying circuitry, we would expect 1) infants to adopt the same interlimb coordination when kicking and when placed on a treadmill, and 2) when the interlimb coordination of one movement is changed through training, there should also be a similar change in the interlimb coordination of the other movement. To test these hypotheses we quantified the interlimb coordination patterns expressed by infants while kicking and while on a moving treadmill belt. We also had a few infants

practice one coordination pattern in a weight-bearing position for a few weeks and examined the effects in weight-bearing (i.e., on the treadmill) and kicking in supine. In accordance with our first hypothesis, 82% of infants showed statistically similar interlimb coordination for the two behaviors. However, the findings from the practice part of the study did not match our second hypothesis. Four weeks of practice resulted in the expression of the practiced interlimb coordination on the treadmill in all cases. Yet, the practiced coordination was not always seen in kicking. These findings suggest that infant kicking and stepping may share underlying neuronal circuitry, but the effect of practice will require more detailed study.

3.2 Materials and Methods

Subjects

Subjects were recruited by a nurse through community parent/infant groups in Edmonton. Infants around 6 months of age were preferentially recruited. Typically these infants are content to play in a supine or prone position as they are not yet mobile. It is difficult to elicit kicking movements in mobile infants since they do not like to stay in a supine or prone position. Moreover, for the training part of the study, infants of this age group are less likely to impose other forms of practice by crawling or walking with support. Sixty-four infants in good health participated. Nine of these infants were assigned to either a group that practiced a specific weight-bearing activity (i.e., either stepping or hopping) or to a control group. All nine infants attended a second testing session about 4 weeks later. A parent provided written informed consent at the time of testing. Study procedures were

approved by the Health Research Ethics Board of the University of Alberta and Capital Health, Edmonton.

Experimental Procedures

Prior to testing parents were asked standardized questions concerning their infant's play habits. For example, they were asked what positions the infant plays in (i.e., supine, prone, sitting), whether the infant was crawling, and how frequently the infant plays in a weight-bearing activity, such as a jolly jumper or exersaucer.

Interlimb coordination of the legs was studied during kicking and weight-bearing on a treadmill for all infants, and also during air-stepping for infants who showed this behavior during testing. Air-stepping is a stepping-like movement seen when infants are held upright in the air with no contact between their feet and a supporting surface (Yang et al., 1998). The parents and a researcher played with the infant during testing since infants are more likely to perform rhythmic movements of the legs when they are excited (Thelen, 1985). Kicking was elicited from the infants followed by stepping/hopping on a treadmill and air-stepping. One to 2 trials of each rhythmic behavior were attempted. Trials lasted 0.5 - 3 minutes and rests of 1 - 2 minutes were given between trials.

Kicking occurred in 1 of 3 ways depending on the preference of the infant: 1) lying supine on a play mat, 2) lying prone on a play mat, or 3) sitting on the edge of a small bench, upper body supported by a researcher and the legs dangling over the edge of the bench. To elicit stepping/hopping, the infants were held under the arms by a researcher

over a split-belt treadmill (model INFSBT-FP, designed and built by R Gramlich and S Graziano, University of Alberta, Edmonton, AB). A split-belt treadmill was used because it is equipped with separate force plates for each belt allowing accurate measurement of vertical forces for each leg. The force data is not used in this study, and thus, will not be discussed further. The 2 treadmill belts were always set at the same speed, usually between 0.1 - 0.4 m/s. The infants were allowed to support as much weight as possible without the legs collapsing into flexion. Air-stepping was attempted by holding the infants under their arms in a vertical position about 30 centimeters above a surface. The researcher's forearms were supported on a platform when holding the infants during the stepping and air-stepping trials to limit imposition of movements on the infant.

To investigate whether motor experience in weight-bearing influences the interlimb coordination expressed on the treadmill and in non-weight-bearing activities, 5 parents agreed to practice one type of coordination in weight-bearing with their infants at home for 4 weeks (practice group). An infant was selected for the practice group if he/she appeared to show a preference for one type of interlimb coordination at the first visit. Parents were asked to practice the coordination type that their infant did not show a preference for in a weight-bearing position, for a minimum of 5 minutes, 2 times daily. This practice frequency and duration has been effective for eliciting stepping in infants (Yang et al., 1998). At the first visit, a researcher instructed the parent on how to practice stepping or hopping with his/her infant. Parents were also asked to avoid practice or play of the opposite coordination type (i.e., the parent was asked to reduce or preferably

eliminate jolly jumper use if the infant was practicing stepping). A researcher phoned parents weekly to check the frequency of practice. At the beginning of the second visit the parent demonstrated how he/she had been practicing either stepping or hopping with his/her infant, so that we could confirm what they were doing at home. Practice was deemed successful if the infant actively performed the practice movement. Infants who were not actively performing the desired movement were not included in the training part of the study.

Four additional infants were also seen a second time. These infants maintained their usual play pattern and served as controls. The control group was included to determine changes that might naturally occur in a 4 week period because of growth and maturation. If there was any change in play habits, as indicated by parent responses to the standardized questions, the infant was not included as a control. Testing at the second visit was identical in procedure to the first visit for infants in the practice and control groups.

Instrumentation

Right and left knee movements were measured with twin-axis electrogoniometers (Penny and Giles, Biometrics Ltd., Blackwood Gwent, UK). The goniometer arms were placed along the long axes of the femur and tibia. Two infants were observed to kick mainly from the hips; therefore, the goniometers were instead placed over their hip joints bilaterally (goniometer arms aligned with the midline of the trunk and long axis of the femur).

The testing session was videotaped (30 frames/second, Canon Elura 50). The camera faced either the left or right side of the infants to record movement in the sagittal plane. The leg facing the camera was the reference leg. The infants wore black leotards. White adhesive markers were placed on the reference leg at the midline of the trunk above the iliac crest, greater trochanter, lateral knee joint line, lateral malleolus and head of the fifth metatarsal. Markers were also applied to the contralateral leg over the medial knee joint line, medial malleolus and medial aspect of the great toe. Surface EMG recordings were collected from most infants. However, the EMG data is not presented in this study and thus, will not be discussed further.

The video and analog signals were synchronized by a digital counter that emitted a light signal and a pulse at 1 Hz. The EMG signals were rectified and high-pass filtered at 10 Hz. Rectified EMG and goniometer signals were amplified, low-pass filtered at 30 Hz, and converted from analog to digital form at 250 Hz (Axoscope, Axon Instruments Inc., Foster City, CA). For back-up purposes, all raw signals were recorded on video tape (A.R. Vetter, Redersburg, PA).

Analysis

After the testing session the video footage was reviewed and sequences of good data were identified. Sequences were defined as at least 2 sequential hip and knee flexion-extension movements in both legs (change in joint angle $\geq$20 degrees in both directions, pause in movement must have been less than 1 second). An infant was included for further analysis if he/she performed at least 15 kicks and 15 steps or hops on the treadmill

in total. Air-stepping data was included if the infant demonstrated at least 15 air-steps in addition.

A customized software program (Matlab, MathWorks, Inc., Natick, MA) was used to quantify the phase lag of the 2 legs for all movements. A cycle was defined as the onset of knee or hip flexion to the subsequent onset of knee or hip flexion (Figure 3.1a, X). Phase lag was defined as the delay in time from the onset of the cycle in the reference leg to the onset of the cycle in the contralateral leg, expressed as a percentage of the cycle in the reference leg. A phase lag of 0 – 20.0% or 80.0 – 100% was considered synchronous interlimb coordination, whereas a phase lag of 20.1 – 79.9% was considered alternate coordination.

Statistics

Due to the cyclical nature of the behaviors studied we used circular statistics (Batschelet, 1981), which have been used in other biological studies to describe rhythmic movements (Graham, 1977; Berkowitz and Stein, 1994; Butt and Kiehn, 2003). Using circular statistics, the phase lag is represented as an angle in a circle. A phase lag of 50% = 180° and 0% = 0°, which is equivalent to 100% = 360°. For descriptive purposes, the mean vector angle and mean vector length (Batschelet, 1981) were calculated for each rhythmic movement performed by an infant. The mean vector length ($0 \leq r \leq 1$) is a measure of dispersion with $r = 0$ indicating a random distribution (Batschelet, 1981). Kuiper's test, a two-sample test of significance adapted for circular statistics from Kolmogorov and Smirnov's test (Batschelet, 1981), was used to identify significantly different phase lag

distributions. A comparison of the phase lag distribution for non-weight-bearing (i.e., kicking) and that for weight-bearing (i.e., treadmill stepping and/or hopping) activity was performed for every infant. The number of infants with statistically similar distributions was totaled, as was the number of infants with different distributions. Using these totals, the sign test (Moore and McCabe, 1999) was used to assess the statistical significance of the number of infants with similar distributions of phase lag. An independent t-test was used to compare the mean ages of the infants with similar and different distributions. The following additional comparisons of phase lag distributions were performed for some infants: 1) weight-bearing on the treadmill and air-stepping, 2) kicking and air-stepping, 3) kicking at the first and second visits, and 4) weight-bearing on the treadmill at the first and second visits. The interlimb coordination adopted by an infant when air-stepping was compared to that shown when weight-bearing on the treadmill to assess whether the treadmill preferentially induces an alternate interlimb coordination. Significance was set at $p<0.01$ for all statistical tests due to the large number of tests performed. All mean values reported are accompanied by one standard deviation.

3.3 Results

Thirty-four of the 64 infants tested met the inclusion criteria for analysis (age range 3.2 – 9.7 months, mean age $\pm$ standard deviation = 6.4 $\pm$ 1.5 months, 19 males and 15 females). Six of these infants also provided sufficient air-steps for analysis. Five infants participated in the practice portion of this study (4 males, 1 female). Four other infants participated as controls (2 males, 2 females).

Kicking, stepping, hopping and air-stepping movements all involved alternating cycles of flexion and extension of the knee joints. Figure 3.1 depicts raw data from the goniometer of the reference knee during these movements of an infant who showed alternate interlimb coordination (Figure 3.1a) and one who showed synchronous coordination (Figure 3.1b). Also, some infants showed smooth transitions between alternate and synchronous forms of a rhythmic movement (example in Figure 3.1c).

Weight-bearing and Non-weight-bearing Interlimb Coordination

Most infants showed a preference for either alternate or synchronous interlimb coordination when weight-bearing on the treadmill and when kicking. Figures 3.2a and b show data from single infants with phase lags centered around 50% and 0 or 100%, respectively. Twelve infants (35%) preferred the alternate form (distributions resembled Figure 3.2a) and another 12 (35%) preferred a synchronous coordination (distributions resembled Figure 3.2b). Figure 3.2c depicts data from one infant who did not express a preference for one type of coordination. Four infants (12%) had phase lag distributions like the infant in Figure 3.2c, indicating no preference. Note that the distributions of these infants had short mean vector lengths in comparison to those who showed a preference for one type of coordination (Figure 3.2c versus a and b). The remaining six infants (18%) showed one interlimb coordination in weight-bearing and the other in non-weight-bearing (Figure 3.2d), or they expressed both coordination types for one activity and only one for the other activity. The corresponding cumulative distributions for infants shown in Figure 3.2 are shown in Figure 3.3. Kuiper's test for circular statistics uses the cumulative distributions to assess statistical significance (Figure 3.3d). Twenty-

eight of the 34 infants showed statistically similar distributions of phase lag for weight-bearing and non-weight-bearing movements, while 6 infants showed significantly different distributions. The number of infants with similar distributions of phase lag (i.e., 28 of the 34 infants) was found to be significant ($p = 0.0002$). This suggests that a relationship does exist between the types of interlimb coordination expressed during weight-bearing and non-weight-bearing rhythmic movements.

The mean phase lags expressed when kicking and when weight-bearing on the treadmill for all infants are shown in Figure 3.4a. The means of the 28 infants with similar distributions (filled circles) fall near the unity line. Three of the 6 infants with different distributions also showed similar mean phase lags when kicking and when on the treadmill (Figure 3.4a, open circles). Hence, for these infants the spread in the data resulted in a significant finding (Batschelet, 1981). The mean age of the infants with different distributions in phase lag was significantly greater than the mean age of the infants with similar distributions (8.2 ± 1.3 months and 6.0 ± 1.3 months, respectively).

Air-stepping Interlimb Coordination

The distributions of phase lags for weight-bearing activities on the treadmill and air-stepping were not significantly different from one another for all 6 infants included in this analysis (i.e., if an infant hopped on the treadmill, he/she air-stepped with a synchronous coordination) (Figure 3.4b). This finding suggests that the sensory feedback provided from the moving treadmill belt does not preferentially induce an alternate coordination of

the legs. Also, there were no differences in the distributions of phase lag between kicking and air-stepping in these infants.

Play Habits

Play habits are thought to be largely dependent on gross motor development. For example, sitting was reported as the most popular play position in infants who were sitting independently (usually $\geq$6 months of age). Eleven of the 12 infants who showed a preference for synchronous coordination in weight-bearing and non-weight-bearing activities used the jolly jumper at least 5-7 times/week. In contrast, only 1 of the 12 infants who preferred an alternate coordination jumped in a jolly jumper 5-7 times/week. Three of the 34 infants were crawling (characterized by an alternate coordination of the legs) at the time of testing. One of these infants, who had been crawling for about one month, showed a preference for alternate coordination when kicking and when on the treadmill. The other 2 infants, who had been crawling for about 1 week and were regular jolly jumper users, showed a preference for synchronous coordination.

Practice of an Interlimb Coordination Pattern

Three of the 5 infants in the practice group successfully practiced either stepping or hopping. The other 2 infants were excluded from the practice part of the study because they did not actively perform the practiced movement at the second testing session. Infant FLN successfully practiced hopping (Figure 3.5). During the first testing session FLN showed no preference for either type of interlimb coordination (Figure 3.5a). Following 4 weeks of hopping practice he showed a clear preference for synchronous

kicking and hopping in weight-bearing (Figure 3.5b). The distributions of phase lags during kicking and treadmill activity from FLN's second visit were not significantly different. In contrast, the distributions were significantly different between the first and second visits for both behaviors (* in Figure 3.5).

Infants ZMD and AIB successfully practiced stepping (Figure 3.6). At the first visit both infants, who were regular jolly jumper users, had a preference for synchronous coordination in both weight-bearing and non-weight-bearing activities (Figure 3.6a, c). Jolly jumper use was reduced from 7 times/week to once/week during the practice period for infant ZMD. This infant showed a clear preference for alternate coordination on the treadmill at the second visit (Figure 3.6b) (treadmill distributions from first and second visits were significantly different). Interestingly, ZMD continued to prefer a synchronous coordination for kicking at the second visit. The parent of infant AIB reported a reduction from daily use of the jolly jumper to 4-5 times/week during the practice period. At the second visit infant AIB expressed both alternate and synchronous coordination on the treadmill, a significantly different distribution from that of the first visit (Figure 3.6d). Like infant ZMD, infant AIB continued to show a preference for synchronous kicking at the second visit.

Control Group

Of the 4 infants in the control group, 1 infant was excluded because the infant's parent reported an increase in jolly jumper use from once/week to 5 times/week between the first and second visits. Hence, 3 infants (2 males, 1 female) served as controls. Two of

these infants showed no significant change in the coordination of the legs in weight-bearing and non-weight-bearing activities over a 4 week period (Figure 3.7a, b). One infant (STN) showed a slight change in the coordination of the legs during kicking (* in Figure 3.7c). At both visits STN showed a preference for synchronous kicking. The difference was a change in the leading leg from the left (contralateral) leg in the first visit to the right (reference) leg in the second visit. This difference is not considered physiologically relevant to our discrimination of synchronous versus alternate coordination since the activity clearly remained synchronous.

3.4 Discussion

The major findings of this study were 1) the same interlimb coordination was expressed by the majority of infants (82%) in weight-bearing and non-weight-bearing activities, and 2) the interlimb coordination in human infants is shaped by motor experience. The training induced change in the preferred coordination during weight-bearing, but did not always translate to a change in non-weight-bearing activity.

Methodological Issues

First, it is difficult to obtain a large number of kicks and steps/hops from these young infants since they stay awake and alert for shorter periods than older infants. To address this issue we required at least 15 kicks and 15 steps or hops from an infant for inclusion in the study. However, it is possible that in some infants the coordination pattern recorded was not a full reflection of their actual preference for interlimb coordination.

Secondly, the circular statistics used has limitations. The mean vector angle, which we used to calculate the mean phase lag, is meaningful only for unimodal distributions (Batschelet, 1981). The majority of the phase lag distributions seen in this study were unimodal, however, a few bimodal distributions existed. Hence, the mean phase lag assigned to these bimodal distributions may not have been an accurate representation of the true mean. Also, as demonstrated in Figure 3.7c, Kuiper's test is sensitive to small differences in distribution, such as a change in the leading leg during synchronous movements. Such a difference, seen only in this one infant, is not considered relevant for our discrimination of synchronous versus alternate coordination.

Possibility of Shared Circuitry for Rhythmic Movements of Human Infants

The structure of the pattern generator for rhythmic leg movements in mammals remains largely unknown. Recent findings in the rodent have identified commissural fibres in the spinal cord that are likely part of the pattern generator and responsible for at least some of the coordination between the legs (Kremer and Lev-Tov, 1997; Butt and Kiehn, 2003; reviewed in Kiehn and Butt, 2003). To facilitate discussion, we will use the half-centre model of pattern generation (Lundberg, 1980), with the understanding that it is clearly a gross oversimplification, even for lower vertebrates (reviewed in Stein, 2005). The half-centre model assumes there is a flexor and extensor centre on each side of the spinal cord to control the legs (Figure 3.8a). The flexor and extensor half-centres mutually inhibit each other. The flexor centres on each side can either inhibit each other (as during walking), or excite each other (as during hopping). Since infants tend to show the same type of interlimb coordination in weight-bearing and non-weight-bearing movements, it

suggests at least some shared circuitry for controlling both movements, and that either the mutually-excitatory or inhibitory pathway is favored (possibly stronger synaptic strength because of greater use).

The Effect of Motor Experience on Interlimb Coordination

We expected that training a specific type of interlimb coordination would result in preferentially strengthening one set of commissural pathways (i.e., excitatory or inhibitory), which we thought would be expressed in both weight-bearing and non-weight-bearing activities. This was not always the case. The interlimb coordination expressed on the treadmill changed following training in all three infants, but the interlimb coordination in kicking did not for two of three infants. There are a number of possible explanations. First, it is possible that weight-bearing and non-weight-bearing movements use completely different sets of circuitry, so that training of one does not affect the other. We feel this is unlikely because the two behaviors showed similar responses to sensory input (chapter 2). Moreover, the vast majority of infants showed similar coordination patterns between the legs in the two movements prior to training.

Secondly, perhaps the expression of the trained interlimb pattern required the specific sensory input of load to be expressed, since training occurred exclusively in the weight-bearing position. One could imagine the commissural interaction to be polysynaptic, with sensory pathways that reinforce the interneurons (Figure 3.8b). When this sensory input is missing, the pathway is not easily activated. A similar behavioral phenomenon was reported by Edgerton et al. (1997), who trained spinal cats to either step or stand with

tail stimulation for 12 weeks. Following training, the cats were capable of performing the practiced behavior only if tail stimulation was provided (Edgerton et al., 1997). Thus, training with specific sensory input results in a dependence on that input.

Thirdly, the initial strength of the commissural pathways may be a factor. One subject (FLN) showed a switch in preferred interlimb pattern for both movements following hopping training. This infant did not show a preference for either interlimb coordination at his first visit, in contrast to infants ZMD and AIB, who showed a clear preference for synchronous movements. Hence, it is possible that once a preference is established it is more difficult to alter the interlimb coordination.

Lastly, there was a lack of control over practice conditions. Parents were given instruction on how and when to practice and this information was reinforced through telephone conversations. The frequency and quality of practice could not be guaranteed, however. We also could not control the interlimb coordination expressed by infants during other activities. Viala et al. (1986) encountered a similar problem when training a synchronous coordination of the hindlimbs in spinal rabbits. They found the training effect was slow to develop because the animals were spontaneously adopting an alternate coordination between daily training sessions. To correct this Viala et al. (1986) bound the rabbits' hindlimbs together between training sessions. Perhaps the total length of time exposed to the training activity and other activities performed in the day are important factors.

The Effect of Age and Maturation

Our results do not suggest that the coordination pattern of the legs depends on age. Thelen (1985) reported the interlimb coordination expressed by infants when kicking to depend on age. She indicated that between 1 – 5 months of age infants typically kicked with one leg. The appearance of in-phase/synchronous kicks occurred between 4 – 6 months of age, whereas reciprocal/alternate kicks were observed in infants aged 6 – 9 months. This was not supported by our data. It was interesting, however, that the infants who showed different distributions of phase lag in weight-bearing and non-weight-bearing activities were significantly older than those whose distributions were similar. Older infants typically have a greater repertoire of motor experience. For example, some older infants were crawling at the time of testing. Also, older infants have likely been exposed to a greater range of environments and toys. This may explain the more complicated distributions of phase lag seen in older infants (i.e., one type of coordination expressed for one behavior, but both types expressed for the other behavior).

Concluding Remarks

We cannot say definitively whether weight-bearing and non-weight-bearing rhythmic movements of human infants share underlying neuronal circuitry, however, the similarities in interlimb coordination for these movements provide supporting evidence for this concept. Motor experience appears to influence the interlimb coordination expressed by infants. However, a more rigorous study with a greater number of infants is warranted to fully understand the effects of training in one activity and its transfer to another similar activity.

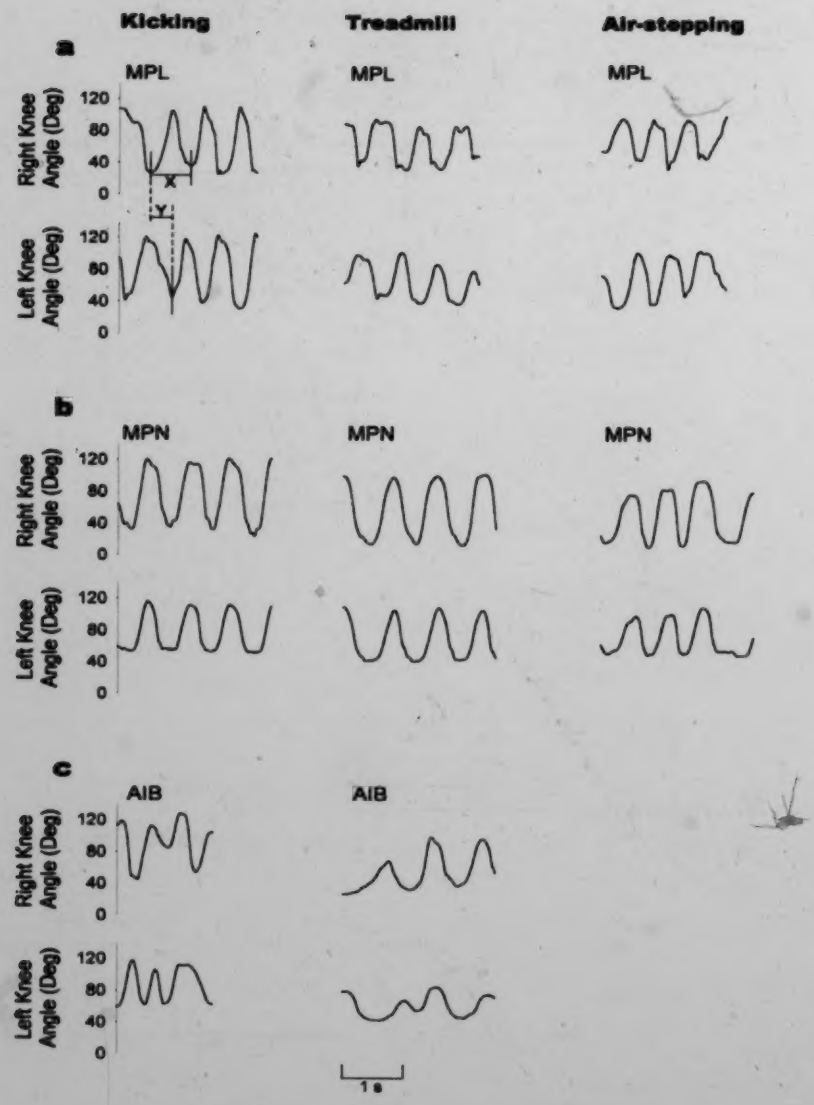

Figure 3.1: Goniometer signals of right and left knees of single infants when kicking (left), on the treadmill (middle) and air-stepping (right). Infant MPL (a) showed an alternate coordination for all 3 movements. MPN (b) showed a synchronous coordination for all 3 movements. AIB (c) showed smooth transitions between alternate and synchronous coordination when kicking and on the treadmill. Increasing angle indicates flexion. For phase lag calculation refer to MPL kicking (a). Solid vertical lines on right (reference) and left (contralateral) signals mark cycle onset. Cycle duration is the difference between 2 successive onsets in the reference leg (X). Onset difference is the difference between onset of contralateral and reference legs (Y). Phase lag=(Y/X)(100%).

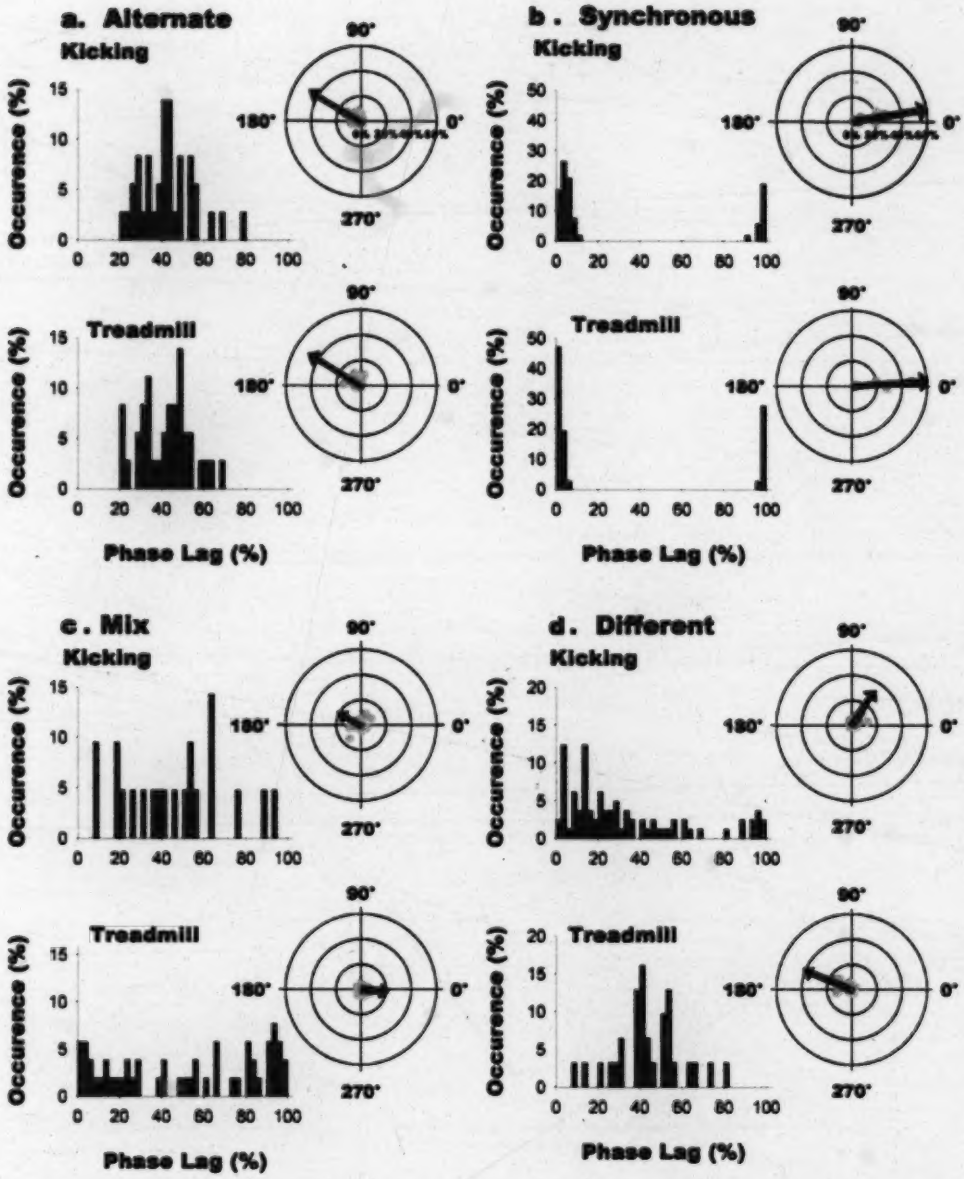

Figure 3.2: Phase lag distributions for single infants. Each distribution is presented as a histogram and as a polar plot (black arrow represents mean vector, each gray dot represents a bin on the corresponding histogram). Preference for alternate coordination for both distributions (a), preference for synchronous coordination for both distributions (b), no preferred coordination for both distributions (c), and differing preferences for coordination for the 2 distributions (d). Phase lag bin size is 2.5%.

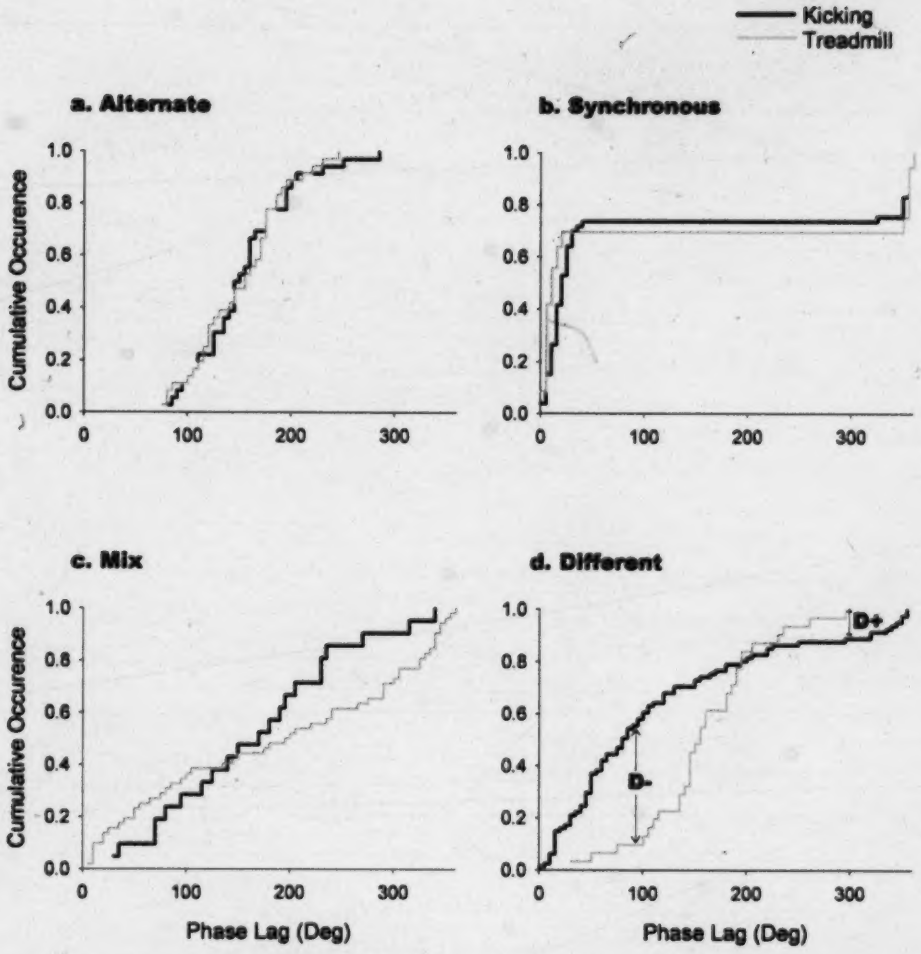

Figure 3.3: Cumulative distributions for phase lag in single infants. Plots show data from the same 4 infants as that shown in Figure 3.2. The cumulative distributions were used for Kuiper's test. Kuiper's test uses the greatest difference between distributions to assess significance (i.e., D- and D+ indicate points where phase lags differ the most, with kicking>treadmill and treadmill>kicking, respectively, for d). The distributions in plot d were significantly different ($p<0.01$). All others were not significantly different. Phase lag bin size is 5^{o}.

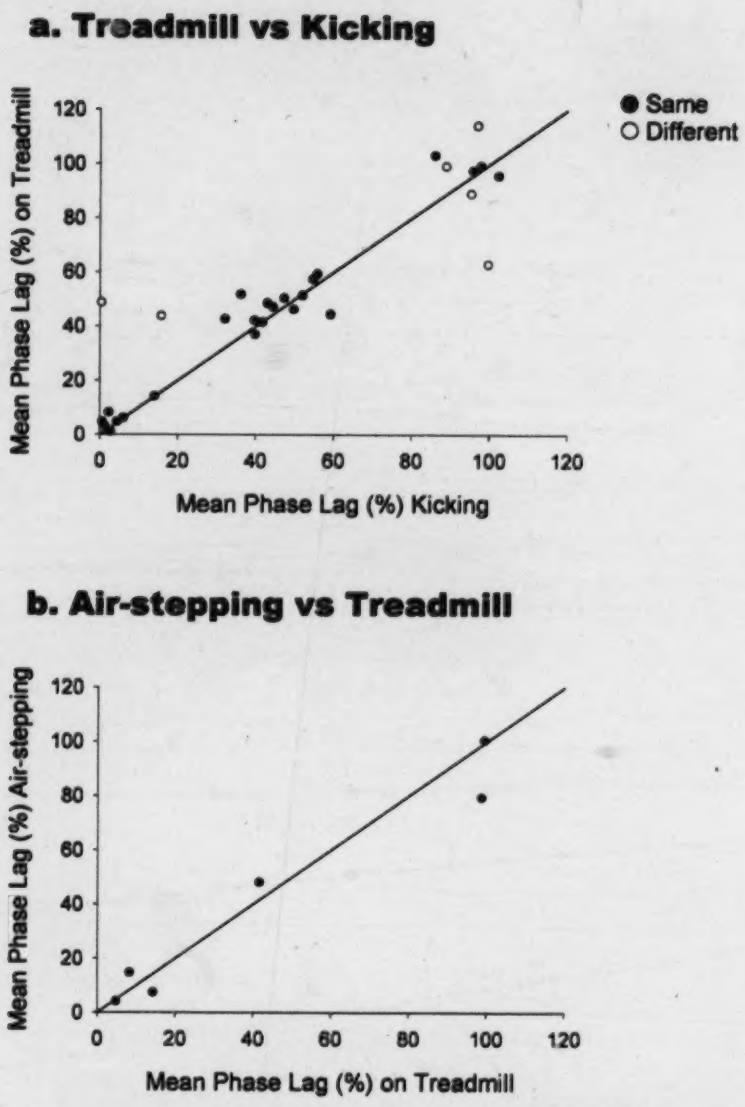

Figure 3.4: Mean phase lag of the legs when kicking versus on the treadmill (a) and when on the treadmill versus air-stepping (b). Each circle represents the average values from 1 infant. Filled circles cluster around 0% and 100% (synchronous coordination) and 50% (alternate coordination). Four infants who showed no clear interlimb preference when kicking and when on the treadmill were excluded from (a) because the means are not meaningful. Three infants in (a) and 1 in (b) had mean values between 0 - 20% for one distribution and between 80 - 100% for the other. Numerically these means appeared very different, but the coordinations were in fact similar, as seen by the description using circular statistics. To resolve this problem, the mean value equal to 0 - 20% was added to 100% to obtain a mean value >100%.

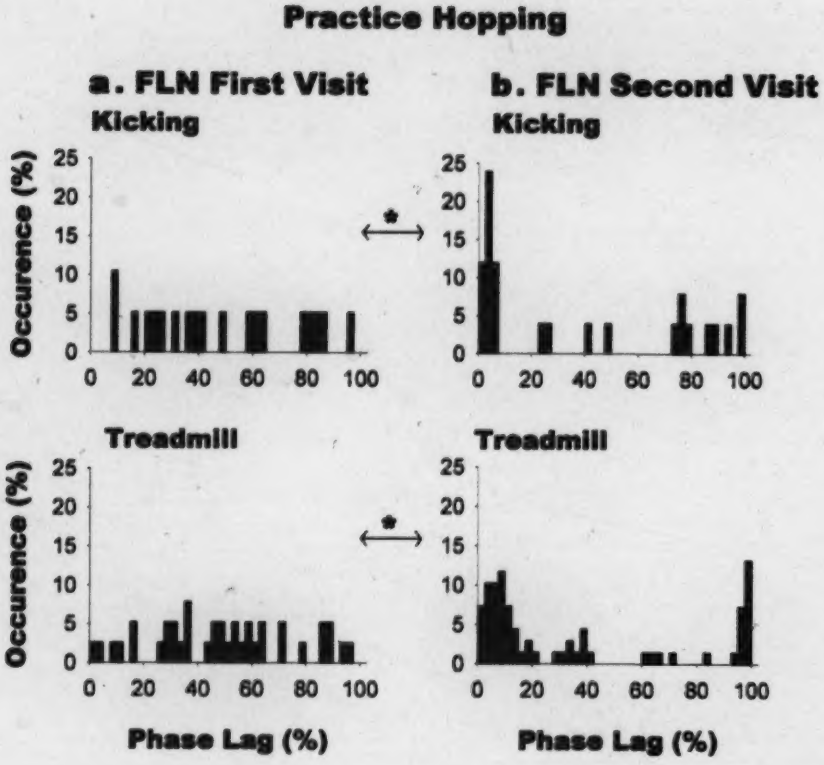

Figure 3.5: Phase lag distributions for infant FLN at his first visit (a) and following 4 weeks of hopping practice (second visit, b). * indicates phase lag distributions that were significantly different ($p<0.01$). Bin size is 2.5%.

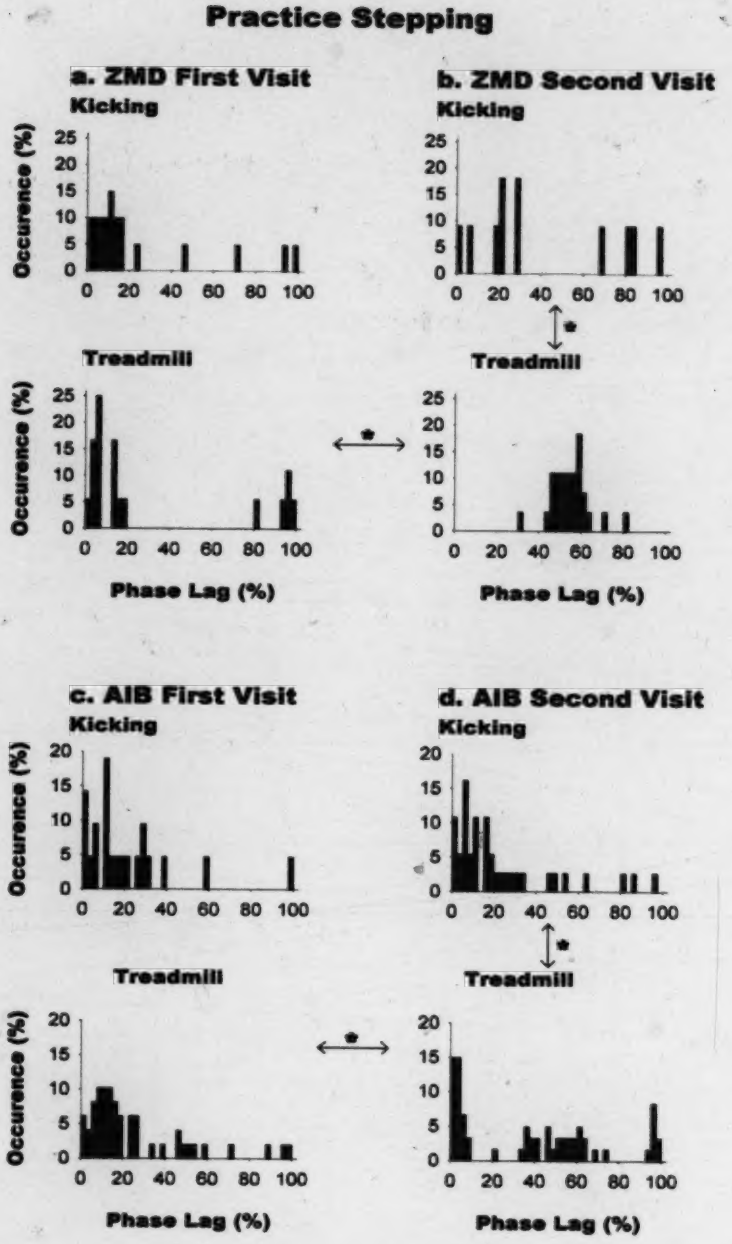

Figure 3.6: Phase lag distributions at the first visit for infants ZMD (a) and AIB (c) and following 4 weeks of stepping practice (ZMD (b), AIB (d)). All conventions are the same as Figure 3.5.

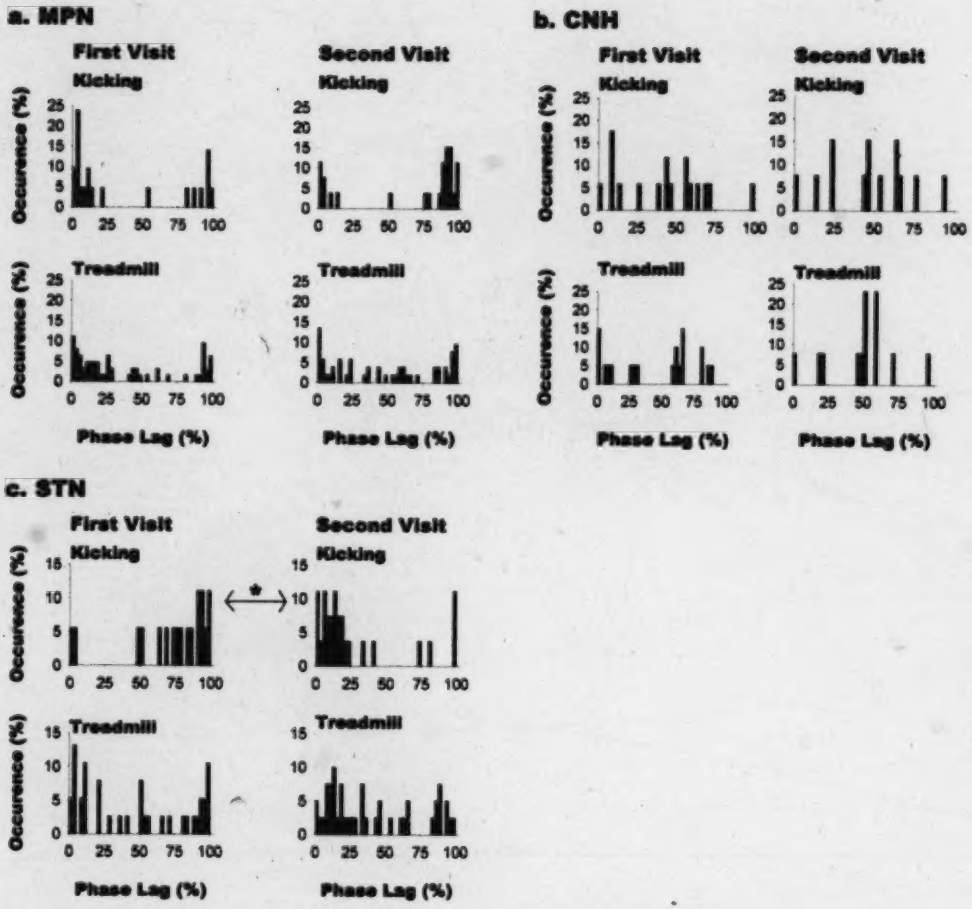

Figure 3.7: Phase lag distributions for infants in the control group, who did not practice and returned for a second testing session. All distributions remained the same except for the kicking pattern in STN (c). The difference in this baby was a switch from leading with the left (contralateral) leg during the first visit to leading with the right (reference) leg in the second visit. Both, however, represent synchronous forms of kicking. All conventions are the same as Figure 3.5.

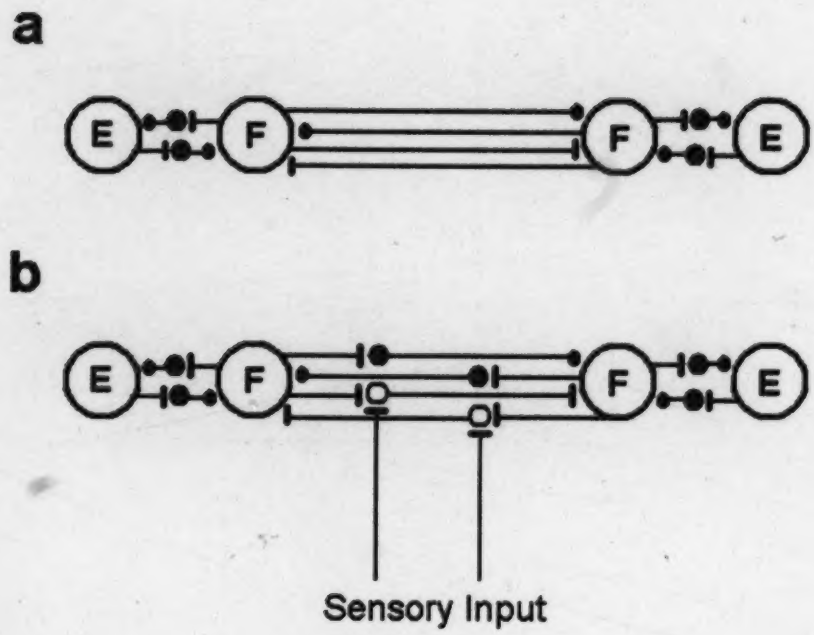

Figure 3.8: A model of the organization of the central pattern generator with sensory input. (a) Half-centre model with flexor and extensor centres controlling the left and right legs. The flexor half-centres mutually excite or inhibit one another. Inhibitory and excitatory commissural interneurons between flexor centres regulate expression of interlimb coordination. (b) Alternative half-centre model in which mutual excitatory and inhibitory connections between the flexor half-centres includes an interneuron. This model can explain the finding that practice with specific sensory input strengthens projections to specific commissural interneurons between flexor half-centres. Sensory input is shown for one set of interneurons only, but similar input is assumed to affect the other set of interneurons. The black dots represent inhibitory connections and the bars represent excitatory connections.

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Chapter 4: Discussion

4.1 Chapter Overview

In this chapter we discuss the findings and implications of the research presented in chapters 2 and 3. We review the major findings of this thesis in section 4.2 and discuss the limitations of using human infants as subjects in section 4.3. In section 4.4 we review the applications of research that examines the behavioral aspects of the central pattern generator (CPG) for locomotion, with emphasis on physical rehabilitation. The future directions of our work are outlined in section 4.5 and concluding remarks provided in section 4.6.

4.2 Major Findings

The work in this thesis provides greater insight into the nature of the human CPG for locomotion through study of spinal/brainstem-mediated control of rhythmic leg movements in human infants. In chapter 2 we compared the phase–cycle relationships of kicking in supine, stepping on a treadmill, kicking against a surface, kicking in sitting, air-stepping and weighted air-stepping. The phase (i.e., flexion or extension) experiencing the greatest load-related afferent feedback (i.e., the powerstroke) was observed to vary more strongly with cycle duration. This finding suggests that the locomotor CPG may not have an inherent asymmetry in the control of extension and flexion phases, but instead can show an asymmetry for either phase or show no asymmetry at all depending on the afferent input it receives at the time of movement. In chapter 3 we demonstrated that the majority of infants express the same type of interlimb coordination in non-weight-bearing (i.e., kicking and air-stepping) and weight-bearing

(i.e., treadmill stepping/hopping) activities. This finding is evidence that the neuronal circuitry controlling rhythmic leg movements in infants may be the same. Motor experience (i.e., play habits) appears to influence which interlimb coordination an infant prefers. We also showed, in a small number of infants, that it is possible to manipulate the preferred coordination through daily motor practice. This finding demonstrates that use-dependent plasticity likely exists in human infants. However, further investigation is warranted to determine whether the effects of training one type of interlimb coordination in a weight-bearing position translate to non-weight-bearing activities.

When interpreted collectively, the findings presented in this thesis suggest that infant kicking, stepping and air-stepping may share underlying neuronal circuitry. The descriptions of the movements in section 2.3 highlight basic similarities in intralimb characteristics of the three movements. For example, all three rhythmic movements are characterized by alternating cycles of flexion and extension. However, intralimb characteristics were not investigated fully in this thesis and a more extensive intralimb analysis would be useful. Range of motion and the relative timing of flexion and extension movements of the hip, knee and ankle joints could be compared for the different rhythmic movements. These intralimb characteristics reflect the motor output of the pattern-generating circuitry and we would expect the characteristics to be similar if the three movements share controlling circuitry. The research presented in chapter 2 also demonstrated that the different rhythmic movements of infants show common responses to afferent input related to load; further evidence for common underlying circuitry. The interlimb characteristics of the three behaviors were investigated in chapter 3. Most

infants expressed the same interlimb coordination when kicking, stepping and air-stepping, which is what would be expected if the behaviors shared neuronal circuitry. Smooth transitions between behaviors (i.e., between air-stepping and stepping and between kicking in sitting and stepping) were frequently observed during data collection for this thesis, but these observations have not yet been studied in detail (see section 4.5). As previously mentioned in section 1.8, smooth transitions, referred to as switches, are evidence of shared neuronal circuitry controlling the two behaviors (reviewed in Stein, 2005). Therefore, like stepping, swimming and scratching in the turtle (Earhart and Stein, 2000; reviewed in Stein, 2005) and walking and hatching in the chick (Bekoff et al, 1987; reviewed in Bekoff, 1992), it seems likely that infant kicking, stepping and air-stepping share a common pattern-generating circuitry.

4.3 Using the Human Infant to Study Central Pattern Generation

In section 1.5 we discussed the use of humans under the age of one year as a model of study for the spinal/brainstem control of human locomotion. There are several reasons why the infant is an attractive model for such study. Much of what we know about the CPG for locomotion has come from study of reduced invertebrate and vertebrate preparations, in which descending and/or afferent input to the spinal cord is removed. Yet, the human nervous system is different from that of lower vertebrates; hence it is ideal to use human models for study. There are few opportunities to study humans in reduced states – individuals with spinal cord injuries (all or some of the descending input is removed depending on the completeness of the injury), individuals with sensory polyneuropathies (removal of some afferent input), and the human infant (limited

descending influence). The advantage of using the human infant over individuals with spinal injuries or sensory neuropathies is that the infant is a healthy model. Injured individuals often present with complicating factors, such as spasticity, muscle atrophy and bowel/bladder dysfunction, that affect their motor behavior. Also, considerable plasticity occurs with time in these injured individuals, causing the nervous system to be in an abnormal state.

However, it is important to discuss the limitations of using the human infant as a model for spinal/brainstem-mediated locomotion. Our current understanding of central nervous system development in humans is based largely on work done in lower vertebrates, such as rodents and chicks. Histological evidence from human fetuses and young children seems to confirm that the sequence of developmental events described in these animals pertains to humans as well (reviewed in Altman and Bayer, 2001). But, we do not know with certainty the timing of developmental events in the human. There is considerable evidence suggesting that the corticospinal tract is not fully myelinated until two years of age (Yakovlev and Lecours, 1967; Brody et al., 1987; Eyre et al., 1991; Khater-Boidin and Duron, 1991; Szelenyi et al., 2003; reviewed in Altman and Bayer, 2001), and it is thought to be the last descending tract to mature (reviewed in Altman and Bayer, 2001). We do not know exactly what descending influences infants under the age of one year experience and this poses a limitation in using the human infant to study the human CPG for locomotion. In addition, we must rely on behavioral observations when using the human infant as a model of study. It is very difficult to obtain good electromyographic (EMG) data from infants because of fat tissue and the high likelihood of crosstalk

between antagonistic muscles. This means we often rely on kinematic data for analyses. Hence, our methods are indirect and we are unable to make any definitive conclusions about the CPG for human locomotion.

4.4 Applications

The work presented in this thesis contributes to our knowledge about central pattern generation for locomotion in the human. It therefore adds to our current understanding of the control of walking, which has a diverse range of therapeutic and non-therapeutic applications. Non-therapeutic applications are seen mainly in the area of robotics. Walking robots are desirable for explorative and military purposes as they can be used to traverse inaccessible or dangerous terrain (reviewed in Delcomyn, 2004). Robots and computer simulations can even further our understanding of the physiological basis of locomotion. Researchers use them to test their hypotheses concerning the control of walking (reviewed in Delcomyn, 2004).

As we learn more about the underlying control of human walking, more effective prosthetics are designed, better walking aids are made, and therapeutic strategies for the recovery of locomotion are improved. Research concerning the CPG for locomotion has had a significant impact on physical therapy for individuals with spinal cord injuries. Body weight-supported treadmill training has become a popular treatment for individuals with incomplete spinal cord injuries. This approach is based on work in cats that demonstrated treadmill training with weight support to be an effective way to train hindlimb stepping following a spinal injury (Lovely et al., 1986; Barbeau and Rossignol,

1987). Treadmill training is thought to activate the spinal circuits (i.e., CPG) involved in locomotion by providing the appropriate rhythmic sensory cues (Dobkin et al., 1995). For example, loading of the legs and extension of the hips are important cues facilitated by treadmill stepping (reviewed in Dietz and Harkema, 2004). Body weight-supported treadmill training was reported to facilitate more normal leg EMG activity during stepping, suggesting training or plasticity of spinal circuits (reviewed in Dietz and Harkema, 2004). The effects of treadmill training translate to functional gains too, as it was shown to be effective at improving overground walking speed and endurance in individuals with incomplete spinal cord injuries (reviewed in Wernig et al., 2000; Harkema, 2001; Barbeau et al., 2002). Treadmill training has also been shown to be a promising therapy for individuals with stroke (Visintin et al., 1998; Sullivan et al., 2002), Parkinson's disease (Miyai et al., 2000, 2002; Pohl et al., 2003) and cerebral palsy (Schindl et al., 2000). Administration of drugs (i.e., noradrenergic agonists) improves the stepping motor pattern of spinal cord-injured individuals during treadmill walking (Fung et al., 1990). This beneficial effect of certain drugs on locomotion was first discovered in spinal cats (Barbeau and Rossignol, 1987) and provides another example of how research is applied to therapeutic practice.

The research presented in this thesis adds to our knowledge of central pattern generation for locomotion in the human. We have demonstrated the important effect of load on the flexion and extension phases of rhythmic movements. This information can be incorporated into computer and robotic models of walking to create more accurate simulations. We have shown that play habits influence the interlimb coordination

adopted by infants. Thus, questions concerning play habits should be included in gross motor assessment of pediatric clients. We have provided supporting evidence for the possibility that multiple rhythmic movements of the legs in humans share underlying neuronal circuitry. In addition, we demonstrated that training a movement in weight-bearing can affect non-weight-bearing movements. Taken together these findings have implications for the rehabilitation of individuals with spinal cord injuries. Initially following an injury these individuals often do not have sufficient cardiovascular stability or stamina to begin upright walking training. However, training walking movements in non-weight-bearing positions could be an effective method of locomotor training in the early stages of a spinal cord injury. Much more research is required to determine whether the effects of training stepping-like movements in non-weight-bearing translate to upright walking, but the research presented in this thesis is a good start.

4.5 Future Work

For future work we plan to expand upon some of the findings presented in this thesis. In chapter 3 we showed that it is possible to successfully train stepping or hopping in three infants. We would like to study motor training in human infants in greater depth. We plan to test whether training one type of interlimb coordination in non-weight-bearing affects an infant's preferred coordination in weight-bearing. This training protocol is more relevant to therapy than the protocol tested in this thesis (i.e., train in weight-bearing and observe effects in non-weight-bearing movements). As mentioned in section 4.4, if training stepping-like movements in supine benefits stepping ability in weight-bearing, effective walking therapy can begin during the acute phase of a spinal cord

injury. Also, the training protocol we used for the study reported in chapter 3 could be improved. The parents were administering the practice; therefore we could not guarantee the frequency or quality of practice. We would like to adopt a more rigorous practice protocol and include a greater number of subjects. Viala et al. (1986) trained synchronous and alternate interlimb coordination in spinal rabbits using a bicycle-like apparatus. We plan to build a similar apparatus that would permit rhythmic leg movements, but in only one of the two interlimb forms, synchronous or alternate. The infant will play with the apparatus in the supine position and receive positive feedback, such as activation of a musical mobile, when he/she kicks with his/her legs (the mobile will be coupled to the practice apparatus). A biofeedback system has been effective in eliciting specific leg movements from human infants (Angulo-Kinzler et al., 2002). This will allow us to see whether training in a non-weight-bearing position (i.e., lying supine) affects movement in a weight-bearing position (i.e., on a treadmill). We may find that the effects of training are task-specific and that non-weight-bearing training does not affect weight-bearing movements. Indeed, Edgerton et al. (1997) demonstrated that motor learning in spinal cats is task-specific (discussed in section 1.11). It would also be interesting to implement a cross-training paradigm in infants similar to that used by Edgerton et al. (1997) in spinal cats. Four weeks of stepping training, which would likely result in the expression of an alternate interlimb coordination on the treadmill (reported in section 3.3), could be followed by four weeks of hopping practice. Would hopping training abolish the expression of an alternate coordination between the legs on the treadmill?

In this thesis we commented that we have observed infants to make smooth transitions between two rhythmic movements and between alternate and synchronous forms of a movement. We also suggest that interlimb phase lags around 30% and 70% may represent a blending, or hybrid, of alternate and synchronous forms. We have previously discussed the significance of the two types of blends, switches and hybrids (introduced in section 1.8). We would like to study switches and hybrids of rhythmic movements in the human infant in detail. What pairings of behaviors show smooth transitions? We could examine many rhythmic movements – kicking, stepping, air-stepping, typical quadruped crawling and other forms of crawling (i.e., scooting, bear crawling, commando crawling). How frequently do switches occur? Are there examples of hybrid behaviors? If so, they should be described. Such a study could provide further supporting evidence that the same CPG for locomotion controls all rhythmic leg movements in human infants.

4.6 Concluding Remarks

There are three main findings reported in this thesis. First, not all rhythmic leg movements performed by human infants show an extension asymmetry. Load-related afferent feedback influences the phase-cycle relationship. The phase (i.e., flexion or extension) experiencing the greater resistance or load preferentially varies with changes in cycle duration. This phenomenon is seen in other reports in the literature and provides a unifying explanation of the control of phase and cycle durations in animal locomotion. The fact that the same principle applies to a variety of rhythmic leg movements suggests that the control of these movements must share considerable circuitry. Secondly, the possibility of shared circuitry is also supported by our finding that infants express similar

interlimb relationships when non-weight-bearing (i.e., kicking) and when weight-bearing on a moving treadmill. Lastly, motor experience influences the interlimb coordination expressed by infants, as demonstrated by the manipulation of interlimb coordination through practice. The findings reported in this MSc thesis contribute to our growing understanding of the control of locomotion in humans – a research quest that has been ongoing for over a century. However, each piece of information gained brings researchers closer to fully understanding the complexity underlying this relatively simple motor task.

4.7 References

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